TREATMENT DEVICE USING OZONE GAS

Publication number: JP3139364

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Classification:

- international: ***A61M37/00; A61K33/00; A61M35/00; A61P25/04;*** **A61M37/00; A61K33/00; A61M35/00; A61P25/00;** (IPC1-7): A61K33/00; A61M37/00

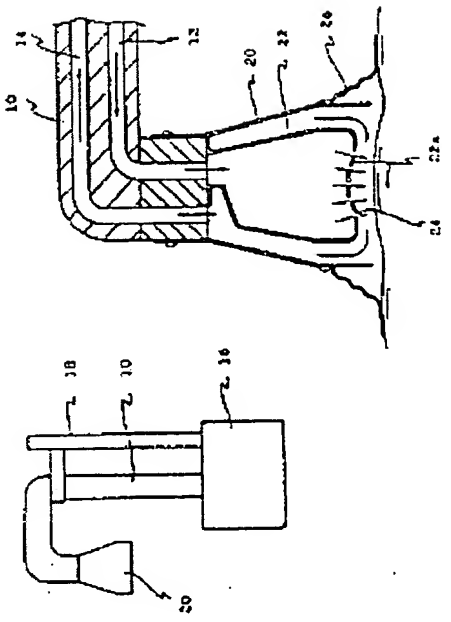
- European:

Application number: JP19890277943 19891025

Priority number(s): JP19890277943 19891025

Abstract of **JP3139364**

PURPOSE:To simplify handling at the time of treatment and to lightly apply treatment with ozone gas by blowing ozone gas against the lesion to bring the same into contact with the affected part. CONSTITUTION:The ozone gas formed in an ozone apparatus 16 is fed in a blowoff body 22 through a feed-out flow passage 12 to be emitted to the outside from a blowoff port 24. Next, the ozone gas emitted to the outside comes into contact with the lesion to be returned while sucked toward the ozone apparatus 16 from a suction flow passage 14. The ozone gas blown off from the blowoff body 22 is intercepted by a hood 20 so as no to leak to the outside and effectively recovered while sucked on the side of the suction flow passage 14. When the hood 20 is used so that the lesion is allowed to approach the end edge part of the hood 20 in an almost contact state, an almost hermetically closed space is formed and effect preventing the outflow of the ozone gas to the outside is enhanced.



⑲日本国特許庁(JP)　⑪特許出願公開

⑫公開特許公報(A)　平3−139364

⑤Int. Cl.⁵	識別記号	庁内整理番号	㊸公開　平成3年(1991)6月13日
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審査請求　有　請求項の数 1　(全4頁)

㊹発明の名称　オゾンガスを用いた治療器

㉑特　願　平1−277943
㉒出　願　平1(1989)10月25日

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明細書

1．発明の名称　オゾンガスを用いた治療器

2．特許請求の範囲

1．オゾンガスを生成し送出する送出機構及びオゾンガスを回収するための回収機構を備えたオゾン装置と、

一端が前記送出機構、回収機構のそれぞれに連絡する送出用流路および吸引用流路を備えた流路管と、

該流路管の他端に前記送出用流路および吸引用流路を内部に開口させて接続されるフードと、

該フード内において前記送出用流路あるいは吸引用流路に連通され、オゾンガスを通流させる通流孔が穿設された中空体と

を備えることを特徴とするオゾンガスを用いた治療器。

3．発明の詳細な説明

(産業上の利用分野)

本発明はオゾンガスを用いた治療器に関する。

(背景技術)

オゾンガスは酸化力がきわめて強い気体であり、その酸化作用によって殺菌、脱色、脱臭効果を有することが知られている。従来のオゾンガスの利用としては、この殺菌、脱色、脱臭作用を利用したものがほとんどで、たとえば以下のような利用例がある。

殺菌作用を利用するものとして、室内の環境浄化、食品の殺菌防腐、上水道、プールの殺菌、傷口の消毒、器具類の消毒。脱色・脱臭作用を利用するものとして、水道水の脱臭、下水処理場の脱臭、ホテル・病院などの空気浄化。この他、酸化、分解、表面活性化、二重結合の切断反応等を利用する例がある。

このようにオゾンガスの利用としては一般には殺菌、脱臭作用を利用した工業的利用がほとんどであるが、オゾンガスの殺菌性に着目して傷口の消毒や水虫を治療する例、肌に刺激を与えて血行をよくするなどの美顔器としての利用なども考えられている。

ところで、本出願人はオゾンガスの利用について研究した結果、上記の殺菌性等の作用に加えてオゾンガスが顕著な鎮痛効果を有することを見出した。すなわち、捻挫などの炎症を起こしている部位にオゾンガスを接触させることによって、炎症による痛みが効果的に軽減できることが見出された。この鎮痛効果はオゾンガスを患部の外部からあてるだけで作用するものであり、腰の痛み、関節部の痛み、打撲の痛みなど各種の痛みを和らげることに有効に作用することが確かめられた。

このオゾンガスの鎮痛効果は、殺菌性、脱臭性等のオゾンガスの効果とは異なる作用であり、この鎮痛効果を利用することによって医療分野に効果的に利用することが可能となる。

本発明は上記のオゾンガスの鎮痛効果に着目してなされたものであり、その目的とするところは、腕、足などの患部の治療に簡単に利用できる効果的なオゾンガスを用いた治療器を提供しようとするものである。

（課題を解決するための手段）

本発明は上記目的を達成するため次の構成をそなえる。

すなわち、オゾンガスを生成し送出する送出機構及びオゾンガスを回収するための回収機構を備えたオゾン装置と、一端が前記送出機構、回収機構のそれぞれに連結する送出用流路および吸引用流路を備えた流路管と、該流路管の他端に前記送出用流路および吸引用流路を内部に開口させて接続されるフードと、該フード内において前記送出用流路あるいは吸引用流路に連通され、オゾンガスを通流させる通流孔が穿設された中空体とを備えることを特徴とする。

（作用）

オゾンガス装置において生成されたオゾンガスは送出用流路をとおってフード内の中空体に送出され通流孔から吐出される。フードは患部に向けて吐出されたオゾンガスが外部に漏れないように遮蔽し、フード内に開口する吸引用流路によってオゾンガスを吸引して回収する。中空体を吸引用流路に接続した場合は送出用流路から患部に向けてオゾンガスが吐出されるとともに中空体から吸引されて回収される。

（実施例）

以下本発明の好適な実施例を添付図面に基づいて詳細に説明する。

第１図は本発明に係るオゾンガスを用いた治療器の一実施例を示す説明図である。

図で１０はオゾンガスを送排気するための流路管で、内部にオゾンガスを送出するための送出用流路１２およびオゾンガスを吸引して排気するための吸引用流路１４が形成されている。流路管１０の基部は第２図に示すようにオゾン装置１６に連結され、前記オゾンガスの送出用流路１２はオゾン装置のオゾンガス送出機構に、前記オゾンガス吸引用流路１４はオゾンガスの吸引機構にそれぞれ接続されている。

流路管１０は、第２図に示すように、オゾン装置１６上に立設され上部においてＬ字形に折曲するが、折曲位置においてスタンド１８により固定支持される。

流路管１０の先端には先端側が太径となる筒形のフード２０が、下向きに開口するよう取り付けられている。

フード２０は第１図に示すように、流路管１０の流出用流路１２および吸引用流路１４の端部をフード２０内で開口させて取り付けられ、前記流出用流路１２に中空のシェル状に形成された中空体としての吹出し体２２が連通して固定される。吹出し体２２は送出用流路１２との連結部から先側が徐々に拡径し、吹出し体２２の外周面とフード２０内面との間に若干の間隔をあけてフード２０開口部側に延出する。

フード２０の開口面に面する吹出し体２２の吹出し部２２ａは中央部が若干凹状に湾曲するほぼ平面状に形成され、オゾンガスを通流させるための吹出し孔２４が所定数設けられる。

なお、吹出し部２２ａはフード２０の延出位置よりもやや後退した位置に設ける。

続いて、上記実施例の使用方法について説明する。

上記オゾンガスを用いた治療器は腕、足等の患部をフード２０の開口部に近づけ、吹出し部２２ａから吐出されるオゾンガスに患部を接触させるようにして用いるものである。

オゾン装置１６で生成されたオゾンガスは送出用流路１２をとおって吹出し体２２内に送出され、吹出し孔２４から外部に吐出される。外部に吐出したオゾンガスは患部に接触した後、吸引用流路１４からオゾン装置１６側に吸引されて戻る。

第１図に示すように吹出し体２２から吹き出されたオゾンガスはフード２０によって外部に漏れ出ないように遮蔽されるとともに、吸引用流路１４側で吸引することにより効果的に回収することができる。フード２０の端縁部に患部がほとんど接触するぐらいに近づけて使用した場合は、ほぼ密閉空間となりオゾンガスが外部に流出することを防止する効果が高まる。

なお、第１図に示すようにフード２０の開口部の周縁に柔軟なシート材を用いて保護用のスカート２６を垂らすように設けてもよい。この場合は患部をフード２０に近づけた際、スカート２６によってフード２０の周囲が覆われてさらに遮蔽効果を高めることができる。

本実施例のオゾンガスを用いた治療器は患部をフード２０に近づけておくだけでオゾンガスに接触させることができるから、操作上の煩わしさがなくきわめて扱いが簡単であるという特徴がある。そして、患部をフードに近づけてセットすればよいので、腕、足等に限らず腰、背中等適宜箇所に用いることができ、また、たとえばフード２０の向きを可変に形成したり、流路管１０を可撓性に形成したり、伸縮可能に形成したりすることによってセットを容易にすることができ、患者も楽な姿勢で治療を受けることができる。

また、上述したように患部近傍を密封するように遮蔽してオゾンガスを接触させ、外部に漏れるオゾンガスの量を少なくして治療できるから、ある程度濃度の高いオゾンガスも使用でき治療効果を高めることができるという利点がある。

なお、上記治療器ではオゾンガスを回収しつつ使用するが、回収したオゾンガスはオゾン装置１６内で加熱等によって無害化して外部に放出したり、循環再利用したりする。また、一定程度の濃度以下の場合はそのまま外部に放出してもよい。

また、フード２０等は耐オゾン性を有する素材を用いて適宜形状、サイズに設計することができる。また、上記実施例においてはフード２０の内部に配設した中空体に送出用流路１２を連通させたが、中空体を逆に吸引用流路１４に接続してオゾンガスの流れ方向を逆転させ、フード２０の周縁部からオゾンガスを吹き出させ、中空体からオゾンガスを吸引するようにしてもよい。設計によっては、この構成のほうが上記例よりもオゾンガスの外部への流出を抑える効果が高い場合がある。

以上、本発明について好適な実施例を挙げて種々説明したが、本発明はこの実施例に限定されるものではなく、発明の精神を逸脱しない範囲内で多くの改変を施し得るのはもちろんのことである。

（発明の効果）

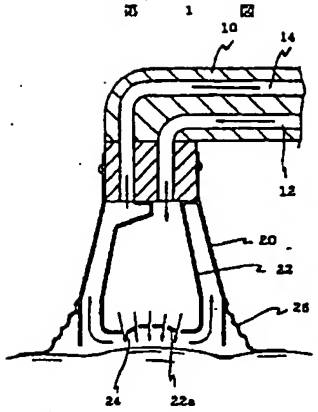
本発明に係るオゾンガスを用いた治療器は、患部に向けてオゾンガスを吹き付けて患部にオゾンガスを接触させるようにしているから、装置としての構成が簡易であり、治療等に際しての取り扱いもきわめて簡単で、手軽にオゾンガスによる治療を受けることができるという効果がある。また、吐出したオゾンガスが外部に漏れないようにしているので、オゾンガスによる刺激臭などの悪影響を抑制することができ、ある程度濃度の高いオゾンガスが使用できて効果的な治療ができる等の著効を奏する。

４．図面の簡単な説明

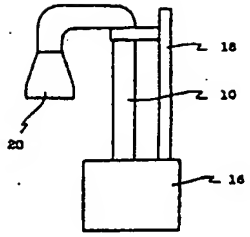
第１図は本発明に係るオゾンガスを用いた治療器の要部を示す説明図、第２図は全体の概略構成を示す説明図である。

１０・・・流路管、　１２・・・送出用流路、
１４・・・吸引用流路、　１６・・・オゾン装置、
１８・・・スタンド、　２０・・・フード、
２２・・・吹出し体、　２２ａ・・・吹出し部、
２４・・・吹出し孔、　２６・・・スカート。

第 1 図



第 2 図

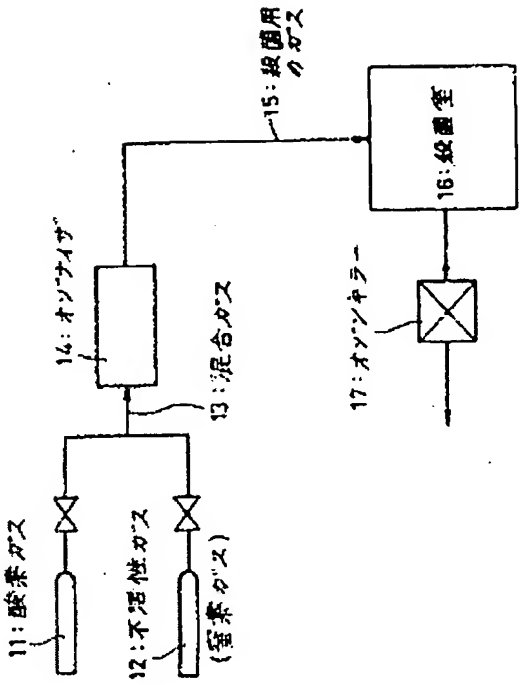


STERILIZATION USING OZONE

Publication number: JP3207365
Publication date: 1991-09-10
Inventor: KAMASE YUKIHIRO; YAMAMOTO KATSUHARU; NAGAHAMA SUNAO; HARA OKITADA
Applicant: ISHIKAWAJIMA HARIMA HEAVY IND
Classification:
- international: ***A61L2/20; C01B13/00;*** **A61L2/20; C01B13/00;** (IPC1-7): A61L2/20; C01B13/00
- European:
Application number: JP19900003949 19900111
Priority number(s): JP19900003949 19900111

Abstract of **JP3207365**

PURPOSE:To perform sterilization within a short time by obtaining large sterilizing power by generating ozone using oxygen gas as raw material gas and subsequently mixing inert gas such as nitrogen gas with the ozone-containing oxygen gas before use.
CONSTITUTION:As raw material gas, a gaseous mixture 13 prepared by mixing oxygen gas 11 and inert gas 12, for example, nitrogen gas is used. The raw material gas wherein the oxygen gas 11 and the nitrogen gas are mixed is sent to an ozonizer 14 and ozone gas is generated by silent discharge. The sterilizing gas containing the ozone gas thus generated is sent to a sterilizing chamber 16 in which an object to be sterilized is received. Therefore, the concn. of oxygen in atmosphere to be sterilized is lowered by the inert gas 12 and the oxidation reaction by the ozone gas is kept. By this method, large sterilizing power can be obtained.



⑲日本国特許庁(JP)　⑪特許出願公開

⑫公開特許公報(A)　平3−207365

⑤Int.Cl.⁵	識別記号	庁内整理番号	⑬公開　平成3年(1991)9月10日
A 61 L 2/20	J	7038−4C	
C 01 B 13/00		6939−4G	

審査請求 未請求　請求項の数 2　(全5頁)

⑭発明の名称　オゾンによる殺菌方法

⑳特　願　平2−3949
㉒出　願　平2(1990)1月11日

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明　細　書

1．発明の名称

オゾンによる殺菌方法

2．特許請求の範囲

(1) ガス状オゾンを用いて殺菌するに際し、酸素ガスと窒素ガスなどの不活性ガスとの混合ガスを原料ガスとしてオゾンを発生させた後、オゾンを含むこれらガスで殺菌するようにしたことを特徴とするオゾンによる殺菌方法。

(2) ガス状オゾンを用いて殺菌するに際し、酸素ガスを原料ガスとしてオゾンを発生させた後、オゾンを含む酸素ガスに窒素ガスなどの不活性ガスを混合して殺菌するようにしたことを特徴とするオゾンによる殺菌方法。

3．発明の詳細な説明

〔産業上の利用分野〕

この発明は、オゾンを用いて行う殺菌方法に関し、殺菌力の増大により食品容器など自動殺菌に好適なものである。

〔従来の技術〕

従来からオゾン（オゾンガス）を用いて殺菌を行うことが行われており、万一残留しても気体となって拡散されるなど人体に安全なことから、例えば飲料水の殺菌などに利用されている。

このようなオゾンによる殺菌を行う場合には、原料ガスとして純粋酸素（濃度99.7%程度の酸素ガス）を原料ガスとして用い、これをオゾン発生器に送ってオゾンを発生させ、発生したオゾンを殺菌用の容器などに送り、容器内の試料を殺菌するようにしている。

このようなオゾン殺菌を行う場合、殺菌用の容器内のオゾン濃度を高めることが殺菌能力に最も大きな影響があると考えられていただめ、できるだけ純度の高い酸素ガスを原料ガスとして高濃度のオゾンを作り出し、高濃度のオゾンを用いて殺菌することが考えられていた。

［発明が解決しようとする課題］

ところが、最近の研究によれば、容器内のオゾン濃度がある濃度以上の状態では、オゾン濃度よりも容器内でのオゾンの酸化反応が支障なく行われるかが、オゾンによる殺菌の良否に大きな影響があることが知得された。

そこで、このようなオゾンの酸化反応が支障なく進行するようにしたオゾンによる殺菌方法の開発が望まれている。

この発明は、かかる従来技術の課題に鑑みてなされたもので、大きな殺菌力を得て短時間に殺菌することができるオゾンによる殺菌方法を提供しようとするものである。

［課題を解決するための手段］

上記従来技術が有する課題を解決するため、この発明のオゾンによる殺菌方法は、ガス状オゾンを用いて殺菌するに際し、酸素ガスと窒素ガスなどの不活性ガスとの混合ガスを原料ガスとしてオゾンを発生させた後、オゾンを含むこれらガスで殺菌するようにしたことを特徴とするものである。

また、この発明のオゾンによる殺菌方法は、ガス状オゾンを用いて殺菌するに際し、酸素ガスを原料ガスとしてオゾンを発生させた後、オゾンを含む酸素ガスに窒素ガスなどの不活性ガスを混合して殺菌するようにしたことを特徴とするものである。

［作　用］

このオゾンによる殺菌方法によれば、原料ガスとして酸素ガスと窒素ガスなどの不活性ガスとの混合ガスを用い、発生させたオゾンガスとともに、不活性ガスを用いて殺菌するようにしており、不活性ガスによって殺菌すべき雰囲気中の酸素濃度を低下し、オゾンガスによる酸化反応を持続するようにし、大きな殺菌力を得るようにしている。

したがって、酸素ガスと窒素ガスの混合ガスである空気を原料ガスとして用い、発生したオゾンガスを含む空気によって殺菌するようにして大きな殺菌力を得ることができる。

また、このオゾンによる殺菌方法によれば、酸素ガスを原料としてオゾンガスを発生させ、このオゾンガスに窒素などの不活性ガスを加えた混合ガスで殺菌するようにしており、オゾンガスに加えた不活性ガスによって殺菌すべき雰囲気中の酸素濃度を低下し、オゾンガスによる酸化反応を持続するようにし、大きな殺菌力を得るようにしている。

［実施例］

以下、この発明の実施例を図面を参照しながら詳細に説明する。

第１図はこの発明のオゾンによる殺菌方法の一実施例にかかる原理説明図である。

このオゾンによる殺菌方法では、原料ガスとして酸素ガス１１と不活性ガス１２、例えば窒素ガスとを混合した混合ガス１３を用いる。

この酸素ガス１１と窒素ガス１２との混合された原料ガスは、オゾナイザ１４に送られ、無声放電などによってオゾンガスが発生される。ここで発生されるオゾンガスは、例えば６０００～１５０００ppm 程度の濃度とされる。

このオゾナイザ１４を通過した原料ガスは、発生されたオゾンガス（O_3）のほか、原料ガスのままの酸素ガス（O_2）１１と窒素ガス（N_2）１２、さらに窒素酸化物のガス（NOx）が含まれた状態となっている。

こうして発生されたオゾンガスが含まれた殺菌用のガス１５は、殺菌対象物が入れられる殺菌室１６などにオゾン濃度が６０００ppm 程度となるように送られる。

なお、図示省略したが、殺菌用のガスを殺菌室１６に送る前に、必要に応じ、酸素ガスを取除いた状態の殺菌用のガス１５とするようにしても良い。

このため、例えば高純度シリカゲルなどの吸着剤が入れられた吸着塔に送り、冷却した状態でオゾンガスを吸着剤に吸着させ、次いで、冷却した状態で吸着塔内に不活性ガスを供給して酸素を塔外に排出し、しかる後、吸着塔を室温にして吸着されたオゾンを不活性ガスによって殺菌室などに供給するようにすれば良い。

このように所定濃度（例えば６０００ppm 程度）

のオゾンガスに窒素ガス及び窒素酸化物のガスが含まれた状態の殺菌用のガス１５を用いて殺菌するようにすると、殺菌室１６内の酸素濃度が低下し、オゾンの酸化作用に伴なって発生する酸素が窒素ガスと化合することとなり、酸化反応が持続し、殺菌作用が増大する。

殺菌後のオゾンガスはオゾンキラー１７に送られて処理された後、排気される。

このように原料ガスとして酸素ガス１１と窒素ガス１２の混合ガス１３を用いることにより所定濃度のオゾンガスを確保した状態で殺菌効果を増大することが可能となるが、混合ガス１３の割合は、通常純粋な酸度ガス１１として市販されている純度９９．７％の酸素ガス１１に窒素ガスなどの不活性ガス１２を混合して行くことで殺菌効果を増大でき、空気のように、酸素濃度が約２０％で、窒素ガス濃度が約８０％の混合割合の場合にも殺菌効果が増大することがわかっている。

〔実験例〕

原料ガスの違いによる殺菌効果の比較を次のような条件下で行った。

条件：オゾンガス濃度　６０００ppm

供試菌　枯草菌胞子

原料ガスとして純粋酸素ガスと乾燥空気（ＤＰ－－６０℃）を用い、処理時間を１分、２分及び５分に変えて殺菌状態を調べた。

その結果、次の表に示すような結果が得られ、原料ガスの違いにより、殺菌効果が大きくことなることがわかった。

	処理時間（分）		
	１分	２分	５分
酸素ガス	効果なし	効果なし	－－
乾燥空気（DP--80℃）	10^3 殺菌	－－	10^4 殺菌

したがって、従来のように高純度の酸素ガスを原料ガスとして用いることなく、空気を原料ガスとして用いて所定濃度（例えば、６０００ppm 程度）のオゾンガスを発生させ、これによって効率良く、しかも安価に殺菌することが可能となる。

次に、この発明の他の一実施例について、第２図を参照しながら詳細に説明する。

第２図はこの発明のオゾンによる殺菌方法の一実施例にかかる原理説明図である。

このオゾンによる殺菌方法では、原料ガスとして酸素ガス２１のみを用いる。

この原料ガスとしての酸素ガス２１は、オゾナイザ２２に送られ、無声放電などによってオゾンガスが、例えば６０００～１５０００ppm 程度の濃度で発生される。

このオゾナイザ２２を通過した酸素ガスには、発生されたオゾンガス（O_3）のほか、原料ガスのままの酸素ガス（O_2）が含まれた状態となっている。

この発生されたオゾンガスと酸素ガスが含まれたガスには、不活性ガス２３として、例えば窒素ガスが混合されて殺菌用のガス２４とされ、このち、オゾンガス濃度が、例えば６０００ppm 程度として殺菌対象物が入れられる殺菌室２５などに送られる。

なお、図示省略したが、発生されたオゾンガスと酸素ガスが含まれたのガスを窒素ガスなどの不活性ガスと混合して殺菌用のガスとする前に、必要に応じ、酸素ガスを取除いた状態の殺菌ガスとするようにしても良い。

このため、例えば高純度シリカゲルなどの吸着剤が入れられた吸着塔に送り、冷却した状態でオゾンガスを吸着剤に吸着させ、次いで、冷却した状態で吸着塔内に不活性ガスを供給して酸素を塔外に排出し、しかる後、吸着塔を室温にして吸着されたオゾンを混合すべき窒素ガスなどの不活性ガスによって殺菌室などに供給するようにすれば良い。

このように所定濃度（例えば６０００ppm 程度）のオゾンガスを発生させた後、窒素ガス２３を混合した状態の殺菌用のガス２４をつくり、これを

用いて殺菌するようにすると、殺菌室２５内の酸素濃度が低下し、オゾンの酸化作用に伴なって発生する酸素が窒素ガスと化合することとなり、酸化反応が持続し、殺菌作用が増大する。

殺菌後のオゾンガスはオゾンキラー２６に送られて処理された後、排気される。

このように所定濃度のオゾンを含む酸素ガスに窒素ガスを混合して用いることにより殺菌効果を増大することが可能となるが、混合ガスの割合は、既に説明した原料ガスを酸素ガスと窒素ガスとする場合において、殺菌室に供給される殺菌ガスと同様に、通常純粋な酸素ガスとして市販されている純度９９．７％の酸素ガスに含まれている不純物としてのガス濃度以上に窒素ガスなどの不活性ガスをオゾンガスに混合して行くことで殺菌効果を増大でき、空気のように、酸素濃度が約２０％で、窒素ガス濃度が約８０％の混合割合の場合にも殺菌効果が増大することがわかっていることから、空気と同じように約８０％の窒素ガスを混合するようにしてもオゾンガス濃度が所定濃度であれば、殺菌作用の増大を図ることができる。

このように、酸素ガス２１からオゾンを発生させた後、窒素ガスなどの不活性ガス２３を混合するようにしているので、原料状態で混合する場合に比べ、殺菌室２５内に供給される殺菌用のガス２４中に、窒素酸化物ガス（ＮＯｘ）が存在せず、反応器としてのオゾナイザ２２などに硝酸塩等の付着が起こらず、メンテナンスが容易となる。

したがって、従来のように酸素ガスを原料ガスとして所定濃度のオゾンガスを発生させ、これに不活性ガスを混合するようにするだけで、特に高濃度のオゾンを発生させること無く能率良く、しかも安価に殺菌することが可能となる。

なお、上記実施例では、不活性ガスとして窒素ガスを例にあげて説明したが、他の不活性ガスは勿論のこと、空気中の窒素ガスも含め、不純物を含む広い意味の不活性ガスであっても良い。

また、この発明の要旨を逸脱しない範囲で各構成要素を変更しても良いことは言うまでもない。

〔発明の効果〕

以上、実施例とともに具体的に説明したようにこの発明のオゾンによる殺菌方法によれば、原料ガスとして酸素ガスと窒素ガスなどの不活性ガスとの混合ガスを用い、発生させたオゾンガスとともに、不活性ガスを用いて殺菌するようにしているので、不活性ガスによって殺菌すべき雰囲気中の酸素濃度を低下し、オゾンガスによる酸化反応を持続させ、大きな殺菌力を得ることができる。

したがって、酸素ガスと窒素ガスの混合ガスである空気を原料ガスとして用い、発生したオゾンガスを含む空気によって殺菌することが可能となり、大きな殺菌力を得ることができる。

また、このオゾンによる殺菌方法によれば、酸素ガスを原料としてオゾンガスを発生させ、このオゾンガスに窒素などの不活性ガスを加えた混合ガスで殺菌するようにしているので、オゾンガスに加えた不活性ガスによって殺菌すべき雰囲気中の酸素濃度を低下し、オゾンガスによる酸化反応を持続し、大きな殺菌力を得ることができる。

また、この場合には、オゾンを発生させる際に不活性ガスの酸化物などが生成されることがなく、反応器などのメンテナンスが容易となる。

４．図面の簡単な説明

第１図はこの発明のオゾンによる殺菌方法の一実施例にかかる原理説明図である。

第２図はこの発明のオゾンによる殺菌方法の他の一実施例にかかる原理説明図である。

１１：酸素ガス、１２：不活性ガス（窒素ガス）１３：混合ガス、１４：オゾナイザ、１５：殺菌用のガス、１６：殺菌室、１７：オゾンキラー、２１：酸素ガス、２２：オゾナイザ、２３：不活性ガス（窒素ガス）、２４：殺菌用のガス、２５：殺菌室、２６：オゾンキラー。

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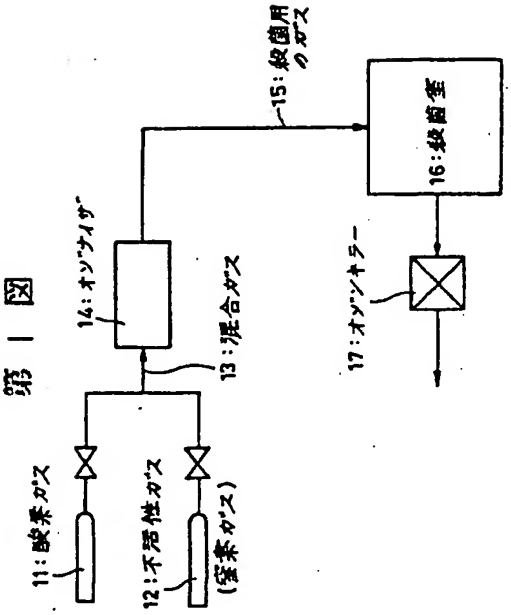
（ほか　１　名）

第1図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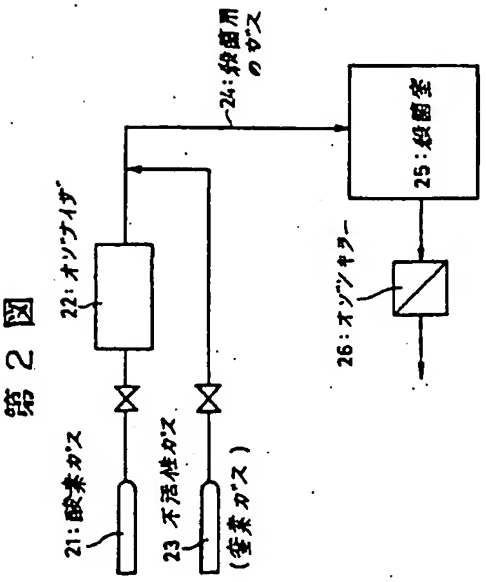
12:不活性ガス
(窒素ガス)
13:混合ガス
14:オゾナイザ
15:殺菌用のガス
16:殺菌室
17:オゾンキラー

第2図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22:オゾナイザ
23 不活性ガス
(窒素ガス)
24:殺菌用のガス
25:殺菌室
26:オゾンキラー

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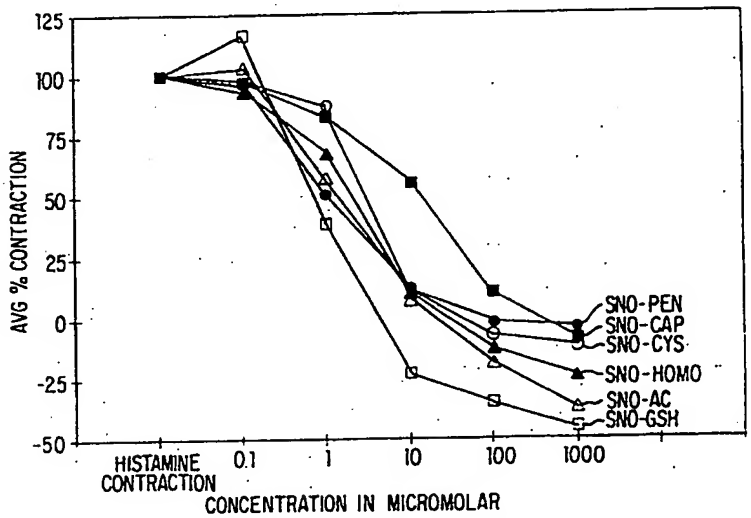

(57) Abstract

S-nitrosothiols exert a potent relaxant effect, mediated by guanylate cyclase, upon non-vascular smooth muscle. Such types of smooth muscle include airway, gastrointestinal, bladder, uterine and corpus cavernosal. Thus, S-nitrosothiols may be used for the treatment or prevention of disorders associated with relaxation of smooth muscle, such as airway obstruction, and other respiratory disorders, bladder dysfunction, premature labor and impotence. Additionally, S-nitrosothiols may be used to alleviate smooth muscle contraction and spasm, and thus facilitate procedures involving diagnostic instrumentation, such as endoscopy, bronchoscopy, laparoscopy and cystoscopy.

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SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
US	United States of America

S-NITROSOTHIOLS AS SMOOTH MUSCLE RELAXANTS AND THERAPEUTIC USES THEREOF

Cross-Reference to Related Application

This application is a continuation-in-part of U.S. Application Serial No. 676,691, filed March 29, 1991.

Background of the Invention

This invention was made with government support under R01-HL40411, HL43344, and R04870, awarded by The National Institutes of Health. The government has certain rights in the invention.

Field of the Invention

This invention relates to the use of low molecular weight S-nitrosothiols, such as S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-homocysteine, S-nitroso-cysteine, S-nitroso-penicillamine and S-

nitroso-captopril, to relax non-vascular smooth muscle. Types of smooth muscle include airway, gastrointestinal, bladder uterine, and corpus cavernosum. The invention also relates to the use of S-nitrosothiols for the treatment or prevention of disorders which involve non-vascular smooth muscle, such as respiratory disorders, gastrointestinal disorders, urological dysfunction, impotence, uterine dysfunction or premature labor. The invention also relates to the use of S-nitrosothiols to ameliorate smooth muscle contraction or spasm and thus, facilitate diagnostic or therapeutic procedures, such as bronchoscopy, endoscopy, laparoscopy, and cystoscopy.

Brief Description of the Background Art

The endothelium secretes a vascular relaxing factor, known as endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide (NO), or a closely related derivative thereof. (Palmer *et al., Nature 327*:524-526 (1987); Ignarro *et al., Proc. Natl. Acad. Sci. USA 84*:9265-9269 (1987)). Under physiologic conditions, however, NO is exceedingly unstable, reacting essentially instantaneously with oxygen, superoxide anion, and redox metals (Lancaster *et al., Proc. Natl. Acad. Sci. USA 87*:1223-1227 (1990); Ignarro *et al., Circ. Res. 65*:1-21 (1989); and Gryglewski *et al., Nature 320*:454-456 (1986)). This fact has lead to the supposition that, in order to exert its effect on vascular smooth muscle, NO must be stabilized *in vivo* in a form that preserves its biological activity.

S-nitrosothiols (RS-NO) are adducts that form readily under physiologic conditions from the reaction of NO with reduced low molecular weight thiols (Oae *et al., Org. Prep. Proc. Int. 15(3)*:165-198 (1983)). These compounds have half-lives that are significantly greater than that of NO and, like EDRF, possess vasorelaxant activity that is

mediated through activation of guanylate cyclase (Kowaluk *et al., J. Pharmacol. Exp. Ther. 256*:1256-1264 (1990); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989); and Ignarro *et al., J. Pharmacol. Exp. Ther. 218(3)*:739-749 (1981)).

The relaxant effect of S-nitrosothiols on blood vessels, and the mechanism by which this effect is exerted, is reasonably well understood in the art. However, the role of NO, or involvement of the guanylate cyclase pathway in non-vascular smooth muscle is not as clearly understood.

In the lung, pulmonary endothelial cells, macrophages and polymorphonuclear leukocytes are potential sources of NO or RS-NO. However, the role of NO and its metabolites in regulation of airway tone is not known, and the few available reports on the efficacy of NO and EDRF in relaxation of airway smooth muscle are conflicting (Shikano et al, J. Pharmacol. Exp. Ther. 243:55-59 (1987); Shikano *et al, Br. Journal Pharmacol. 92*:483-485 (1987)). Furthermore, in the lung, the high ambient concentrations of oxygen and other oxygen-free radicals predispose to rapid inactivation of NO (Furchgott R.F. *et al., I. Endothelium-Derived Relaxing Factors and Nitric Oxide*; eds. Rubanyi G.M., pp. 8-21 (1990); Gryglewski, R.J. *et al., Nature 320*:454-456 (1986)).

Non-vascular smooth muscle is present in numerous organ systems throughout the body, and has a vital role in the physiological function of these systems. For example, airway smooth muscle plays a critical role in constriction and dilation of bronchi. In the gastrointestinal tract, the sphincter of Oddi, a smooth muscle connection between the bile duct and duodenum, provides tonic contraction which serves to prevent reflux of duodenal contents into the pancreatic and bile ducts, and promotes filling of the gall bladder. In addition, esophageal (sphincters and body), intestinal and colonic motility is regulated by smooth muscle. Smooth muscle of the bladder body, bladder base, and proximal urethra plays an

important role in urological function, and erectile function is mediated by relaxation of corpus cavernosal smooth muscle.

In summary, the relaxation kinetics of non-vascular smooth muscle are very important in numerous physiological systems. Moreover, a variety of significant clinical disorders occur, which involve contraction, spasm, or failure to achieve the necessary relaxation of smooth muscle. Examples of such disorders include airway obstruction (i.e., asthma, bronchitis and emphysema), bladder dysfunction, gastrointestinal muscle spasm (i.e., irritable bowel syndrome, achylasia, dumping disorders), and impotence. Thus, a clinical need exists for pharmacological agents which can treat or prevent such disorders by inducing relaxation of the affected smooth muscle.

SUMMARY OF THE INVENTION

This invention is based on the discovery by the inventors that S-nitrosothiols exert a potent relaxant effect on non-vascular smooth muscle. This concept lead the inventors to the discovery that S-nitrosothiol compounds may be used as a therapeutic modality in disorders which involve smooth muscle relaxation.

The invention is directed to an S-nitrosothiol compound which has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

The invention is also directed to an S-nitrosothiol compound which has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

The invention is also directed to an S-nitrosothiol compound which has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20 and Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl; wherein aryl includes benzyl, naphthyl, and anthracenyl groups.

The invention is also directed to the use of S-nitrosothiols for the treatment or prevention of disorders associated with relaxation of smooth muscle, such as airway obstruction, gastrointestinal spasm, bladder dysfunction and impotence. The invention is also directed to the use of S-nitrosothiols to alleviate smooth muscle contraction and spasm, and thus facilitate procedures involving diagnostic instrumentation such as endoscopy and bronchoscopy.

In particular, this invention is directed to a method for relaxing airway smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal. The S-nitrosothiol compound may be selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril. The S-nitrosothiol compound may be selected from the group consisting of a compound having the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

The invention is also directed to an S-nitrosothiol compound which has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

The invention is also directed to an S-nitrosothiol compound which has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20 and Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl; wherein aryl includes benzyl, naphthyl, and anthracenyl groups.

The invention is also directed to a method for treatment or prevention of respiratory disorders by administering a therapeutically effective amount of S-nitrosothiol compound to an animal. Respiratory disorders include obstructive lung disease, emphysema, asthma, bronchitis, fibrosis, excessive mucus secretion, obstruction of air flow, and lung disorders resulting from post-surgical complications.

The invention is also directed to a method for relaxing gastrointestinal smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is also directed to a method for ameliorating contraction or spasm of gastrointestinal smooth muscle associated with endoscopic procedures, by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is also directed to a method for relaxing corpus cavernosum smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is directed to a method for the treatment or prevention of impotence by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is also directed to the administration of said S-nitrosothiol compounds for the methods of the invention, as part of the pharmaceutical composition comprising a pharmaceutically acceptable carrier.

The invention is also directed to the methods of the invention wherein the pharmaceutical composition containing the S-nitrosothiol compound is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or intranasal delivery.

The invention is also directed to a method for relaxing bladder smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

The invention is also directed to a method for relaxing uterine smooth muscle by administering a therapeutically effective amount of an S-nitrosothiol compound to an animal.

Brief Description of the Figures

FIGURE 1: Inhibition of the Sphincter of Oddi by administration of S-nitroso-N-acetylcysteine.

FIGURE 2: Inhibition of duodenal motility by administration of S-nitroso-N-acetylcysteine.

FIGURE 3: Side-by-side comparison of the relaxant effect of specific S-nitrosothiols on guinea pig tracheal muscle.

FIGURE 4: Dose-dependent relaxant effect of specific S-nitrosothiols on guinea pig tracheal muscle contracted with 3 μM, as compared to the reactant and NO.

a: S-nitroso-glutathione
b: S-nitroso-cysteine
c: S-nitroso-homocysteine
d: S-nitroso-N-acetylcysteine
e: S-nitroso-penicillamine
f: S-nitroso-captopril

FIGURE 5: Relaxant activities of S-nitroso-N-acetylcysteine (A) and S-nitroso-captopril (B) determined against contractions induced by leukotriene D_4, histamine and methacholine.

FIGURE 6: The course of relaxation induced by S-nitroso-N-acetylcysteine (5×10^{-6}M) over 60 minutes.

FIGURE 7: The relaxation response to S-nitroso-glutathione in guinea pig airway (A) and rabbit aorta (B).

FIGURE 8: Tracheal relaxant effects of S-nitroso-N-acetylcysteine, isoproterenol, and theophylline.

FIGURE 9: Inhibition of tracheal relaxation to S-nitroso-N-acetylcysteine by hemoglobin and methylene blue.

FIGURE 10: Cyclic GMP determinations in tracheal rings incubated with 100 μM S-nitroso-N-acetylcysteine.

FIGURE 11: Comparison between the relaxant effect of S-nitroso-glutathione and nitrite upon human tracheal smooth muscle.

FIGURE 12: Comparison between the relaxant effect of S-nitroso-glutathione and glutathione upon human tracheal smooth muscle.

FIGURE 13: Comparison between the relaxant effect of S-nitroso-N-acetylcysteine and N-acetylcysteine upon human tracheal smooth muscle.

FIGURE 14: Tracheal relaxant effects of theophylline, isoproterenol, S-nitroso-N-acetylcysteine, and S-nitroso-glutathione.

FIGURE 15: Cyclic GMP response to S-nitroso-N-acetylcysteine in human airways.

Description Of The Preferred Embodiments

Background

The invention is based on the discovery by the inventors that S-nitrosothiols relax non-vascular smooth muscle, and possess unique and different relaxant activities, kinetic properties and membrane permeability, and thus, may be used to treat or prevent disorders which involve non-vascular smooth muscle.

In one embodiment, the term "S-nitrosothiol" refers to a compound which is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-pantathoeine derivatives, S-nitroso-penicillamine and S-nitroso-captopril.

In another embodiment the term "S-nitrosothiol" refers to particular novel S-nitrosothiol compounds synthesized by the inventors, for use as smooth muscle relaxants. The compounds represented by the general formula of $CH_3(CH_2)_xSNO$ are long carbon-chain lipophilic nitrosothiols. The compounds represented by the general formula of $HS(CH_2)_xSNO$ are S-nitrosodithiols, possessing an additional thiol group. The compounds represented by the general formula of $ONS(CH_2)_xY$ are S-nitrosothiols which possess other functional groups, in addition to the thiol.

The invention is related to the discovery that S-nitrosothiol compounds relax non-vascular smooth muscle. As a result, these compounds may be used to treat or prevent those pathophysiologic conditions which result from, or involve, constriction of smooth muscle, or those which necessitate therapeutic intervention to achieve smooth muscle relaxation.

One embodiment of the invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol to an animal to relax airway smooth muscle. The term "airway smooth muscle" refers to the smooth muscle lining the bronchi or tracheal region. The inventors have demonstrated that S-nitrosothiols exert a potent relaxant effect upon airway smooth muscle.

As a result of this potent relaxant effect exerted by S-nitrosothiols, these compounds may be administered as therapeutic agents for the treatment or prevention of respiratory disorders.

The term "respiratory disorder" refers to any impairment of lung function which involves constriction of airways and changes in blood gas levels or lung function.

For example, airway obstruction constitutes a respiratory disorder which occurs as a result of acute pulmonary impairment or obstructive lung disease. Severe airway obstruction may ultimately result in life-threatening respiratory failure. Airway obstruction occurs in patients with chronic obstructive lung diseases, such as emphysema and bronchitis. These patients often experience recurrent episodes of respiratory failure as a result of severe airway obstruction. Emphysema can result in significant disability due to dyspnea, extreme restriction of physical activity, and mortality.

Airway obstruction also results from asthma, a disorder characterized by increased responsiveness of the tracheobronchial tree to various stimuli, and which leads to generalized airway constriction manifested by dyspnea, cough and wheezing. Asthma sufferers often experience acute exacerbations of bronchoconstriction, which may be life-threatening.

Another obstructive lung disease, cystic fibrosis, results from abnormal exocrine gland function. Clinical manifestations include excessive mucous secretion, hypertrophy of bronchial glands, infection,

and inflammatory and structural changes in the airways which lead to obstruction and ventilation-perfusion imbalance.

Acute respiratory failure may result not only from obstructive disease, but also as a consequence of airway constriction secondary to pneumonia, thromboembolism, left ventricular failure and pneumothorax. Acute respiratory failure may also result from ventilation-perfusion imbalance.

A critical component in the treatment of airway obstruction involves the use of pharmacologic agents to remove secretions and reverse airway constriction. The most commonly used bronchodilatory agents are beta-agonists, such as isoproterenol, given by inhalation or subcutaneous injection, and methylxanthines, such as theophylline, given orally or by infusion.

The margin of safety for theophylline administration is relatively narrow. The minimum therapeutic concentration in plasma is 6 to 10 μg/ml, and unacceptable symptoms of toxicity usually appear at or above 20 μg/ml. Still higher concentrations can lead to serious central nervous system toxicity, with long-term ingestion of theophylline being a predisposing factor in such toxicity. Moreover, because the clearance of theophylline is influenced by genetic, developmental and environmental factors to a significant degree, it is necessary to titrate the dosage cautiously against clinical observations of beneficial or toxic effects, with periodic determination of the concentration of the drug in plasma (Gilman A.G., *The Pharmacological Basis of Therapeutics,* Pergamon Press, New York, (1990)).

Isoproterenol, a non-selective β-agonist, produces cardiovascular side effects such as palpitations, sinus tachycardia and more serious arrhythmias. In addition, tolerance to this drug may result from overuse (Gilman A.G., *The Pharmacological Basis of Therapeutics,* Pergamon Press, New York, (1990)). This characteristic reduces its usefulness in patients

with chronic obstructive disease who rely heavily on frequent use of bronchodilators. It has now been demonstrated that β agonists may have long term deleterious effects which result in aggravation of asthma, and ultimately change the natural history of the disease. Consequently, the American Thoracic Society no longer recommends treatment with β agonists as first line therapy in mild asthma (Expert Panel Recommendation, *New England Journal of Medicine, 325:*425-426 (1991)).

The use of S-nitrosothiols for the treatment of airway obstruction provides significant advantages over current methods of treatment. The use of S-nitrosothiols eliminates the untoward side effects associated with β-agonists and methylxanthines.

Furthermore, because all current treatment methods act by way of cAMP, S-nitrosothiols satisfy the need for bronchodilators which act by way of cGMP. This is important because current evidence provided by the inventors demonstrates a role for cyclic GMP in regulation of airway tone in humans (See Example 1). In addition, cyclic GMP agonists act synergistically with cyclic AMP agonists to provide bronchodilation, not obtainable by individual agents.

S-nitrosothiols also potently inhibit platelets and neutrophils, which have been implicated in the pathogenesis of asthma. S-nitrosothiols provide nitric oxide in its biologically relevant form, which is critical because the bioactivity (relaxant activity) of nitric oxide in airways depends upon the form in which it is delivered.

Finally, the inventors have demonstrated that S-nitrosothiols mediate the activity of vasoactive intestinal peptide (VIP), an important airway relaxant. This reinforces the importance of S-nitrosothiols in regulation of airway tone. Deficiency in the effect of VIP is a causal factor in the pathogenesis of asthma. Administration of S-nitrosothiols replenishes the mediator itself rather than a less biologically active derivative.

S-nitrosothiols are also suitable for direct instillation into the lungs by bronchoscopic means. This topical administration permits titration of dose, eliminates the untoward side effects of systemic therapy, and enables the use of combination therapy, involving a topical S-nitrosothiol in conjunction with a systemic agent, in problematic cases. This topical therapy would also facilitate endoscopy by suppressing the cough reflex and associated bronchospasm.

An important component in the treatment of airway obstruction is the removal of airway mucous. Thus, airway obstruction often necessitates the administration of a mucolytic agent in conjunction with the bronchodilator. N-acetylcysteine, more commonly known as "Mucomist", is one such agent. S-nitroso-N-acetylcysteine possesses both mucolytic and bronchodilator capabilities.

With respect to combined bronchodilator-mucolytic agents, the mucolytic activity of the compound depends upon the amount of thiol which is preserved after NO delivery. Thus, S-nitrosothiol compounds which contain more than one thiol (dithiol compounds) are particularly suitable for achieving mucolysis. In addition, the long-chain lipophilic S-nitrosothiols which contain more than one thiol are advantageous as mucolytic agents because they have a free thiol, and their lipophilic property facilitates penetration of the compound into the lipid portion responsible for the tenacious viscosity of mucous.

In addition to the treatment or prevention of respiratory disorders, S-nitrosothiols may also be used to facilitate diagnostic and therapeutic bronchoscopy. The term "bronchoscopy" refers to the procedure in which a flexible fiberoptic, or rigid bronchoscope is introduced into the tracheobronchial tree for the purpose of bronchial visualization, lung biopsy or brushings, aspiration of secretions, and delivery of pharmacological agents.

A complication of bronchoscopy, and thus an impediment to the successful completion of the procedure, is bronchospasm. Patients with a prior history of bronchospasm are particularly at risk for acute enhancement of spasm. Thus, S-nitrosothiols may also be used to relax airway smooth muscle and eliminate bronchoscopy-induced bronchospasm.

Another embodiment of the invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol compound to an animal to relax gastrointestinal smooth muscle. The term "gastrointestinal smooth muscle" refers to smooth muscle which is contained in all areas of the gastrointestinal tract. Such areas include, but are not limited to, the esophagus, duodenum, sphincter of Oddi, biliary tract, ileum, sigmoid colon, pancreatic duct and common bile duct. S-nitrosothiols may be used for the treatment or prevention of gastrointestinal disorders. Disorders of the gastrointestinal tract include achylasia (spasm of the lower esophageal sphincter), diarrhea, dumping syndrome, and irritable bowel.

An additional embodiment of the invention relates to the administration of S-nitrosothiols to alleviate contraction or spasm of gastrointestinal smooth muscle, and thus facilitate successful completion of endoscopic procedures. Contraction or spasm of gastrointestinal smooth muscle imposes a technical obstacle which must frequently be overcome in order to enable the clinition to successfully perform endoscopic procedures.

The term "endoscopic procedures" refers to those diagnostic procedures which utilize an instrument which is introduced into the gastrointestinal tract to provide direct visualization of the gastrointestinal tract, for examination and therapeutic purposes. Such purposes include direct visualization, biopsy, access to the common bile duct, fluid aspiration and removal of foreign bodies, polyps, and other lesions. An example of a particular endoscopic procedure is esophagogastro-duodenoscopy, which is utilized for examination of the esophageal lumen,

stomach and duodenum. Another example, endoscopic retrograde cholangiopancreatography (ERCP), enables visualization of the pancreatic duct, common bile duct and the entire biliary tract, including the gall bladder. Further examples of endoscopic procedures are colonoscopy and sigmoidoscopy.

Current methods for alleviating gastrointestinal muscle spasm include the administration of intravenous diazepam, anticholinergics and glucagon, as well as sublingual administration of nitroglycerin. However, these methods produce generalized, systemic effects which persist for a much longer duration than the procedure itself. In addition, nitroglycerin is significantly less effective as a smooth muscle relaxant than S-nitrosothiols, and produces systemic side effects, the most significant of which is hypotension. It is therefore, not used clinically. Clearly, a need exists for a topical smooth muscle relaxant which could be directly instilled into the various regions of the gastrointestinal tract to facilitate both diagnostic and therapeutic endoscopic procedures.

Patient studies, conducted by the inventors, have measured the efficacy of S-nitrosothiols both in facilitating cannulation of the sphincter of Oddi, and in decreasing colon motility to allow for removal of colon polyps. As shown in Figure 1, topical administration of S-nitroso-N-acetylcysteine eliminated duodenal motility. As shown in Figure 2, topical administration of S-nitroso-N-acetylcysteine inhibited the contractile activity of the Sphincter of Oddi, and thus, permitting successful endoscopic cannulation of the sphincter. In addition, administration of S-nitroso-N-acetylcysteine eliminated colon motility to facilitate successful removal of colon polyps. Notably, the relaxant effects were temporary (lasting only for the duration of the procedure), completely reversible and produced no change in systemic blood pressure, heart rate or oxygen saturation. The same type of effects would occur with the use of other cell impermeable S-nitrosothiols, such as S-nitroso-glutathione.

Prior to the present invention, there were no available pharmacological agents which could be applied directly by endoscopic means to exert a direct, immediate, localized, and completely reversible relaxant effect on gastrointestinal smooth muscle. Topical administration of S-nitrosothiols, during endoscopy, eliminates systemic side effects and allows for the use of the lowest effective concentration of the drug.

Administration of S-nitrosothiols obviates the need for sphincterotomy, a procedure which substantially increases the morbidity and mortality of ERCP. In addition, administration of S-nitrosothiols aids in the cannulation and manipulation of the pancreatic duct and biliary tract during therapeutic procedures such as gall bladder cannulation, bile duct stone removal and stent placement, and decreases the incidence of post-ERCP complications, such as pancreatis and cholangitis. Another use of S-nitrosothiols involves the intraoperative injection of these compounds into the gall bladder prior to cholecystectomy to alleviate cystic duct spasm. This would allow for a laparoscopic cholangiogram by providing access to the cystic duct. In addition to the uses discussed above, S-nitrosothiols may also be administered to treat or prevent any other technical problems associated with endoscopy which are known to those in the medical art.

Another embodiment of the invention relates to administration of a therapeutically effective amount of an S-nitrosothiol compound to relax corpus cavernosum smooth muscle. The term "corpus cavernosum" refers to two areas of smooth muscle which lie side by side on the dorsal aspect of the penis, and together with the corpus spongeosum that surrounds the urethra, constitute erectile tissue. This erectile tissue consists of an irregular sponge-like system of vascular spaces interspersed between arteries and veins. Erection occurs when cavernosa smooth muscle relaxation causes a decease in arterial resistance and resulting increase in arterial blood flow to the penis.

Smooth muscle has a critical role in erectile function Thus, another embodiment of the invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol compound for the treatment of impotence. "Impotence" refers to a condition of male sexual dysfunction which is characterized by the inability to obtain or maintain an erection.

Organic causes of erectile impotence, may include endocrine, drug-induced, local injury, neurologic, and vascular. In particular, impotence may result from neurologic blockade caused by such drugs as antihistamines, antihypertensives, psychogenic agents, and anticholinergics. Impotence may also result from neurologic disorders such as interior temporal lobe lesions, spinal cord disorders, and insufficiency of sensory input resulting from diabetic neuropathy. An additional cause of impotence is insufficient blood flow into the vascular network resulting from an intrinsic defect, or from penile trauma.

Currently available methods for treating impotence consist largely of surgical techniques and intracavernosal injections of pharmacological agents. One surgical technique involves the implantation of a penile prosthesis by inserting within the corpora, a small silastic rod. However, this method does not produce full erection and the complication rate is high. Alternatively, an inflatable prosthetic device may be implanted on either side of the corpora, with a connecting reservoir of material placed in the perivascular space. Erection is achieved through the use of pumps which are located in the scrotum.

Intracavernous injection of the smooth muscle relaxant, papaverine has been used to induce erections. However, a significant disadvantage of this treatment method is the need for a painful injection each time an erection is desired. In addition, numerous side effects and complications result from the chronic use of drugs such as papaverine. Clinical reports indicate that a significant proportion of potential candidates refuse these

injections from the onset of treatment. A larger number of patients, even after favorable initial response to the treatment, become increasingly unresponsive or unwilling to continue injections as a means of treatment (Morales *et al.*, *World J. Urol.* *8*:80-83 (1990)).

In general, a significant number of patients who are potential candidates for current methods of impotence treatment refuse initially because of the invasive nature of the treatment, or reject further treatment because of pain, fibrosis, or dissatisfaction with results.

As demonstrated by the discussion above, prior to the present invention, there was a significant need for a less invasive approach to the treatment of impotence. Because they exert a relaxant effect on corpus cavernosal smooth muscle, S-nitrosothiols are particularly well suited for the treatment of impotence.

Administration of S-nitrosothiols results in relaxation of corpus cavernosum smooth muscle, which leads to dilation of the cavernosal arteries and a concommittent increase in blood flow. S-nitrosothiols provide significant advantages in the treatment of impotence over current treatment methods, because they can be administered topically, thereby eliminating the systemic side effects, significant discomfort, fibrosis, and ineffectiveness associated with the currently available, invasive methods of treatment.

Another embodiment of the claimed invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol compound to relax bladder smooth muscle. Bladder smooth muscle includes that of the bladder base, bladder body and proximal urethra. In addition, S-nitrosothiols may be used for the treatment of bladder dysfunction disorders which involve relaxation of bladder smooth muscle. Such disorders include, but are not limited to, problems with bladder filling, volume and continence.

In addition, S-nitrosothiols may be administered to cause relaxation of urethral and bladder base smooth muscle, and thus, facilitate cystoscopic examination of the urinary tract. The term "cystoscopic examination" refers to the introduction of a fiberoptic instrument through the urethra and into the bladder, to achieve visualization of the interior of the urethra and bladder for diagnostic and therapeutic purposes.

Another embodiment of the invention relates to the administration of a therapeutically effective amount of an S-nitrosothiol compound to relax uterine smooth muscle. Increased contractility of uterine smooth muscle precipitates premature labor. Thus, an additional embodiment of the invention relates to the administration of S-nitrosothiol compounds for the treatment or prevention of premature labor.

S-nitrosothiols may also be used to relax fallopian tube smooth muscle. Fallopian tube smooth muscle plays a role in the transport of the egg to the uterus. Thus, S-nitrosothiols may be used to regulate ovum transport, or to facilitate laparoscopic examination of the fallopian tubes, or to facilitate fertilization procedures.

The long-chain lipophilic compounds have unique potential for NO delivery by incorporation into cell membranes, and for accessing the central nervous system (CNS). In the CNS, nitric oxide has been shown to inhibit cell death resulting from ischemic injury, as well as to possess neurotransmitter functions. Membrane permeability achieved by these compounds also provides the unique potential for NO delivery in every organ system. In addition, NO delivery can be regulated by the incorporation of additional functional groups into the molecule. Each functional group, including but not limited to nitrite, nitrate, redox metal, amine, aromatic, and basic amino acids, has its own unique functional aspects which will affect (a) a targeted site of delivery (b) rate of NO release (c) lipophilicity (d) cell permeability (e) duration of action (f) bioactivity and (g) nitrosation potential.

An additional embodiment of the invention relates to the administration of an S-nitrosothiol compound as part of a pharmaceutical composition, comprising a pharmaceutically acceptable carrier, to achieve the physiological effects discussed above.

The pharmaceutical compositions utilized in this invention can be administered by intranasal, oral, enteral, topical, sublingual, rectal, intramuscular, intravenous, or subcutaneous means. The compositions may be administered by medical instrumentation including, but not limited to, bronchoscopic, endoscopic, laporascopic, and cystoscopic means. With respect to the administration of these composition for the treatment of impotence, the term "topical" includes administration in the form of a condom which contains the pharmaceutical composition. Certain S-nitrosothiols, such as lipophilic S-nitrosothiols, are especially suitable for (i.e. lipophilic) incorporation into the condom itself, to provide sustained release of the compound.

The compounds of this invention can be employed in combination with conventional excipients; i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral or intranasal application which do not deleteriously react with the active compounds. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethylcellulose, polyvinylpyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds.

For parenteral application, particularly suitable vehicles consist of solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories. Ampules are convenient unit dosages.

For enteral application, particularly suitable are tablets, dragees or capsules having talc and/or a carbohydrate carrier binder or the like, the carrier preferably being lactose and/or corn starch and/or potato starch. A syrup, elixir or the like can be used wherein a sweetened vehicle is employed. Sustained release compositions can be formulated including those wherein the active component is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

It will be appreciated that the actually preferred amounts of active compounds used will vary according to the specific compound being utilized, the particular compositions formulated, the mode of application and the particular site of administration. Optimal administration rates for a given protocol of administration can be readily ascertained by those skilled in the art, using conventional dosage determination tests conducted with regard to the foregoing guidelines.

According to the present invention, a "therapeutically effective amount" of a pharmaceutical composition is an amount which is sufficient to achieve the desired pharmacological effect. Generally, the dosage required to provide an effective amount of the composition, and which can be adjusted by one of ordinary skill in the art, will vary, depending upon the age, health, physical condition, sex, weight and extent of disease, of the recipient. Additionally, the dosage may be determined by the frequency of treatment and the nature and scope of the desired effect.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are, therefore, to be construed

as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The entire text of all publications cited above and below are hereby incorporated by reference.

EXAMPLES

Example 1. Airway Smooth Muscle Relaxation by S-nitrosothiols

A. Methods

1. Materials

Glutathione, L-cysteine, DL-homocysteine, D-penicillin, hemoglobin (bovine), methylene blue and Medium 199 sets were purchased from Sigma Chemical Co., St. Louis, MO. N-acetylcysteine was obtained from Aldrich Chemical Co., Milwaukee, WI. Captopril was kindly provided by Dr Victor Dzau. Sodium nitrite, histamine and methacholine were purchased from Fisher Scientific, Fairlawn, NJ. Leukotriene D_4 was purchased from Anaquest, BOC Inc., Madison, WI. Antibiotic/antimycotic mixture (10,000 U/ml penicillin G sodium, 10,000 mcg/ml, streptomycin sulfate, 25 mcg/ml amphotericin B) was purchased from Gibco Laboratories, Grand Island, NY. Radioimmunoassay kits for the determination of cyclic GMP were purchased from New England Nuclear, Boston, MA.

2. Preparation of Airways

Male Hartley guinea pigs (500-600g) were anesthetized by inhalation of enflurane to achieve a surgical plane of anesthesia. The trachea were excised and placed in Kreb's-Henseleit buffer (mM): NaCl 118, CKl 5.4, NaH_2PO_4 1.01, glucose 11.1, $NaHCO_3$ 25.0, $MgSO_4$ 0.69, CaCl 2.32, pH 7.4. The airways were then dissected free from surrounding fat and connective tissue and cut into rings 2-4 mm in diameter. The trachea rings were placed in sterile Medium 199 containing 1% antibiotic/antimycotic mixture in an atmosphere of 5% CO_2, 45% O_2, 55% N_2, and kept for up to 48 hours in tissue culture. The experiments were also performed on human airway smooth muscle, isolated by the same method.

3. Preparation of Blood Vessels

New Zealand White female rabbits weighing 3-4 kg were anesthetized with 30 mg/kg IV sodium pentobarbital. Descending thoracic aortic were isolated and placed immediately in a cold physiologic salt solution (Kreb's) (mM): NaCl 118, CCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 12.5, and D-glucose 11.0, pH 7.4. The vessels were cleaned of adherent connective tissue, and the endothelium removed by gentle rubbing with a cotton tipped applicator inserted into the lumen, and cut into 5 mm rings.

4. Preparation of S-nitrosothiols

S-nitrosothiols were prepared at 25°C by reacting equimolar (100 μM) concentrations of reduced thiols with $NaNO_2$ in 0.5 N HCl (acidified $NaNO_2$) as described previously (Kowaluk *et al., J. Pharmacol. Exp. Ther.*

256:1256-1264 (1990); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989); and Ignarro *et al., J. Pharmacol. Exp. Ther. 218(3)*:739-749 (1981)). Solutions turned from clear to various shades of red instantaneously upon product formation, with the notable exception of S-nitroso-penicillamine, which is green.

In this method of synthesis, the reaction of thiols with NO (generated from sodium nitrite) is complete and stoichiometric (Aldred *et al., J. Chem. Soc. Perkin Trans. II*:777-782 (1987); Byler *et al., J. Agric. Food Chem. 31*:523-527 (1983)).

The long-carbon chain lipophilic nitrosothiols, long and short chain S-nitrosodithiols, and S-nitrosothiols with additional functional groups were synthesized by one or more of the following methods: (a) exposure to equimolar N_2O_3 or N_2O_4 in CCl4; (b) exposure to equimolar acidified nitrite; (c) exposure to equimolar bubbled NO gas; (d) exposure to excess cold bubbled NO_2 gas; and (e) exposure to metherolic acid or equimolar $NaNO_2$ diluted in methersol.

The synthesis of S-nitroso-homocysteine has not been previously characterized. This compound displayed the distinct absorption maxima of other S-nitrosothiols at approximately 340 nm and 550 nm (Kowaluk *et al., J. Pharmacol. Exp. Ther. 256*:1256-1264 (1990); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989); and Ignarro *et al., J. Pharmacol. Exp. Ther. 218(3)*:739-749 (1981)). The molar absorptivity of S-nitroso-homocysteine at 547 nm is 16.7 $cm^{-1}M^{-1}$ and correlates well with published values of 16.6 and 16.1, for S-nitro-cysteine and S-nitroso-glutathione, respectively (Kowaluk *et al., J. Pharmacol. Exp. Ther. 256*:1256-1264 (1990)).

Owing to the modest decay of S-nitrosothiols over time, fresh examples were made at hourly intervals and kept at 4°C until use. Solutions were diluted as necessary into physiologic buffer immediately prior to each experiment.

5. Bioassay

Trachea and aortic rings were mounted on stirrups and connected to transducers (model FOT3C Grass) with which changes in isometric tension were measured. Rings were then suspended in 10 cc of oxygenated (95% O_2, 5% CO_2) buffer. Conditions for both the vessel and airway bioassays were established according to standard methodologies as described in Cooke *et al., Am. J. Physiol. 28*:H804-H812 (1989).

In airway experiments, the rings were equilibrated for 60 minutes under a load of 1 gm and then primed twice by exposure to 100 μM methacholine. Tissues were contracted with various agonists at concentrations determined to generate 50% ($\pm$ 16% S.D.) of maximum tone, after which the effects of different thiols and their S-nitrosylated derivatives were assessed. In selected experiments, relaxation responses were determined in the presence of hemoglobin, or after rings had been preexposed to methylene blue for 30 minutes.

In vessel experiments, aortic rings were contracted with 1 μM epinephrine and relaxations were induced with S-nitrosothiols.

6. Cyclic Nucleotide Assays

The mechanism by which S-nitrosothiols relax vascular smooth muscle is felt to be through activation of guanylate cyclase with consequent increase in intracellular cyclic GMP (Ignarro *et al., Circ. Res. 65*:1-21 (1989); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989)). In order to assess this mechanism in airways, tracheal rings in Kreb's-Henseleit solution were exposed to 100 μM S-nitroso-N-acetylcysteine (SNOAC) for 90 seconds. Reactions were terminated by

the addition of ice cold 10% trichloracetic acid and rapid freezing in ethanol-saturated dry ice.

In selected experiments, rings were preexposed to the guanylate cyclase inhibitor, methylene blue (10^{-4} M) for 30 minutes. Tissues were then individually pulverized with a glass (s) homogenizer and centrifuged at 8000 g for 5 minutes. The clear supernatant was extracted with water-saturated ether and assayed for cyclic GMP by radioimmunoassay. Acetylation of samples with acetic anhydride was used to increase the sensitivity of the assay and the determination of recoveries was aided by the use of [^{3}H] cyclic GMP.

Dose-response relationships to SNOAC were obtained in airways contracted with 3 uM histamine, and repeated in the presence of 10^{-4} M hemoglobin, 10^{-5} M methylene blue, and 10^{-4} M methylene blue. Relaxation responses to SNOAC are inhibited by hemoglobin and methylene blue, with the latter in a dose-dependent manner. Cyclic GMP determinations were performed in duplicate for each experiment.

7. Statistics

All results are presented as means ± SEM. Paired samples were compared by the Student's t-test. Dose-response curves were compared by two-way analysis of variance (ANOVA). Values of $p < 0.05$ were considered significant.

Table 1 Inhibitory Concentrations Inducing 50% Relaxation (IC50)	
RS-NO	IC50 mean ± S.D.; x 10^{-6} M)
S-nitroso-glutathione	0.99 ± 2.0
S-nitroso-cysteine	3.2 ± 0.2
S-nitroso-homocysteine	2.1 ± 0.3
S-nitroso-N-acetylcysteine	2.1 ± 0.8
S-nitroso-penicillamine	1.8 ± 0.8
S-nitroso-captopril	20.0 ± 0.7

B. Results and Discussion

The mammalian fraction of sulfur that exists as free sulfhydryl is contained largely in the form of glutathione, cysteine, and homocysteine (Jocelyn, P.C., In *Biochemistry of the SH Group*, Academic Press, London/New York pp. 1-46 (1972)). N-acetylcysteine is a minor metabolite of cysteine that is used for its mucolytic properties in the treatment of airway obstruction. N-acetylcysteine has also received attention within the context of nitrate metabolism and undergoes S-nitrosylation *in* plasma upon treatment with nitroglycerin (Fung *et al., J. Pharmacol. Exp. Ther. 245(2)*:524-531 (1988)). The S-nitrosylated derivatives of these four sulfhydryls comprise the group of biological S-nitrosothiols under investigation.

Captopril and penicillamine are examples of nonbiological low molecular weight thiols, and their S-nitrosylated derivatives have been well characterized (Kowaluk *et al., J. Pharmacol. Exp. Ther. 256*:1256-1264

(1990); Loscalzo *et al., J. Pharmacol. Exp. Ther. 249(3)*:726-729 (1989); and Ignarro *et al., J. Pharmacol. Exp. Ther. 218(3)*:739-749 (1981).

An initial examination of the relaxant activity of each of the biological and nonbiological S-nitrosothiols in guinea pig tracheal rings was conducted. The results are shown in Figures 1 and 2(a)-(f). As demonstrated by dose-response relationships, these compounds are potent airway smooth muscle relaxants, with relaxant effects that are unmatched by equimolar amounts of reactant thiol or NO (generated from $NaNO_2$ alone).

In every case, the dose-response curves for the S-nitrosothiols were significantly different from the dose-response curves for NO and for the individual thiols by two-way ANOVA to $p < 0.001$. Results are presented as mean $\pm$ SEM, ($n = 5$).

With the exception of S-nitroso-captopril (SNOCAP), the S-nitrosothiols revealed comparable bioactivity with IC50s in the range of 1×10^{-6} M (Table 1). SNOAC and SNOCAP were then selected as representative biological and nonbiological S-nitrosothiols for further detailed investigation.

Dose-effect relationships were obtained for SNOAC and SNOCAP using tracheal rings induced to constrict with leukotriene D_4, histamine, and methacholine. As shown in Figure 3, airways exhibited agonist specificity toward S-nitrosothiol-mediated relaxations: S-nitrosothiols were most active for relaxation of leukotriene D_4-induced contractions and progressively less effective with contractions induced by histamine and methacholine. In every case, SNOAC was approximately 10-fold more active in relaxation of airways than SNOCAP. Results are presented as mean $\pm$ SEM ($n=3$-5).

The time course of relaxation to SNOAC is shown in Figure 4. Using a concentration (5×10^{-6} M) selected to induce approximately 50% of the maximal response, maximal relaxation occurred by five minutes and

a significant residual loss of tone was still evident at one hour. In control experiments, airway contractions remained stable over the study period.

These relaxation responses contrast markedly with those generally ascribed to low-molecular-weight S-nitrosothiols. Figure 5 illustrates the notable difference in relaxation kinetics between these tissues. In vascular smooth muscle, the relaxations are rapid and transient, whereas in tracheal smooth muscle, relaxations occur more slowly and persist for a much longer duration.

Figure 6 shows a comparison between the efficacy of SNOAC and isoproterenol or theophylline under identical study conditions. Of the drugs evaluated, isoproterenol was the most active relaxant, however, SNOAC was approximately 50 times more active in relaxation than theophylline. The dose response curves for these agents are each significantly different from each other by two-way ANOVA to $p < 0.01$. Results are expressed as mean $\pm$ SEM (n=3-5).

Hemoglobin and methylene blue are established inhibitors of NO-induced relaxations in vascular smooth muscle (Ignarro *et al., Circ. Res.* *65*:1-21 (1989)). When their effects were examined in airways, hemoglobin and methylene blue each significantly attenuated the actions of SNOAC, as evidenced by rightward shifts in the dose-effect relationships to SNOAC in their presence (Figure 7). The dose-response curves for SNOAC were significantly different from each of the curves derived in the presence of hemoglobin and methylene blue by two-way ANOVA to $p=0.05$. Results are presented as mean $\pm$ SEM (n=3-5).

The biochemical mechanism of action of S-nitrosothiols was further investigated in isolated tracheal rings. As shown in Figure 8, tracheal rings incubated with SNOAC exhibited 4-fold increases in cyclic GMP over basal levels (control). Increases in cyclic GMP were attenuated by pretreatment of tissues with the guanylate cyclase inhibitor, methylene blue (10^{-4}M). Cyclic GMP levels in the presence of SNOAC were

significantly greater than control values ($p < 0.0005$) and levels determined in the presence of methylene blue ($p = 0.05$). Results are presented as mean $\pm$ SEM ($n = 4$-8).

An examination of the relaxant activity of S-nitrosothiols in human tracheal rings was also conducted. The results are shown in Figures 11-15. In particular, Figure 11 shows that S-nitroso-glutathione has a relaxant effect upon human trachea which is significantly greater than nitrite (NO_2). Figure 12 demonstrates that the relaxant effect of S-nitrosoglutathione upon human trachea is significantly greater than glutathione alone. This data underscores the fact that the bioactivity of nitric oxide in airways depends upon the form in which it is delivered. S-nitrosothiols provide efficient delivery of NO in its most bioactive form.

Figure 13 demonstrates that the relaxant effect of SNOAC upon human trachea is significantly greater than that of N-acetylcysteine. As shown in Figure 13, NAC caused significant constriction of the tracheal smooth muscle, which is consistent with the fact that NAC, when given as a mucolytic agent, causes the untoward side effect of bronchospasm. SNOAC not only causes relaxation of airway tissue, but also eliminates bronchospasm.

Figure 14 demonstrates that SNOAC and SNOGSH exert a relaxant effect on airway smooth muscle which is significantly more potent than that of theophylline, and compares favorably with that exerted by isoproterenol.

Experiments were also conducted to assess the cGMP response to SNOAC in human airways. As shown in Figure 15, tracheal rings incubated with SNOAC exhibited 4-fold increases in cyclic GMP over basal levels (control).

Unexpectedly, the relaxation response to low molecular weight S-nitrosothiols in airways differs markedly from that observed in blood vessels. In the latter case, relaxations occur slowly and persist for a much

longer duration. This is most likely attributed to the inherent differences between vascular and nonvascular smooth muscle. There may be additional contributing factors responsible for this heterogeneity. Finally, any disparity among smooth muscle cells in redox state, availability of reducing equivalents, pH, oxygen tension, or any other factor that might influence the stability of the S-NO bond would be predicted to influence relaxation kinetics.

The results also suggests that, in addition to the primary site of obstruction in the lung, the efficacy of nitro(so)-bronchodilators may be determined by the nature of the chemical mediators contributing to bronchoconstriction. In particular, S-nitrosothiols were most effective in this study against leukotriene D_4-mediated bronchoconstriction and progressively less effective against histamine and methacholine-mediated constriction. Thus, regional variation in guanylate cyclase content or activity, the site of obstruction, the form in which the active species of NO is administered, and the nature of the bronchoconstrictor stimuli are all variables which may influence the determination of nitro(so)-bronchodilator efficacy and the importance of guanylate cyclase in regulating airway tone.

In summary, S-nitrosothiols are believed to be important intermediates in the metabolism of organic nitrates and endogenously-derived NO. These compounds are more stable than NO and retain its cyclic GMP-dependent bioactivity in blood vessels.

In the present invention, the inventors have demonstrated that S-nitrosothiols are also potent airway smooth muscle relaxants and mediate their effects through activation of guanylate cyclase. The results indicate that cyclic GMP is an important inhibitory mediator of airway tone, and suggest a mechanism by which the bioactivity of NO is preserved in the presence of high, ambient concentrations of oxygen and oxygen-derived free radicals. The inventors have also demonstrated that S-nitrosothiols

are present in human airway secretions and mediate the airway relaxation caused by VIP. As a result of their potent relaxation effect upon airway smooth muscle S-nitrosothiols have significant pharmacological utility for the treatment of airway obstruction.

WHAT IS CLAIMED IS:

1. A compound having the formula:

$$CH_3(CH_2)_2SNO$$

wherein:

X equals 2 to 20.

2. A compound having the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

3. A compound having the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

4. A method for relaxing airway smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

5. The method of claim 4 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

6. The method of claim 4 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

7. The method of claim 4 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

8. The method of claim 4 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

9. The method of claim 4 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

10. The method of claim 9 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, intramuscular, aerosol, topical or bronchoscopic delivery.

11. A method for treatment or prevention of respiratory disorders, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

12. The method of claim 11 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

13. The method of claim 11 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

14. The method of claim 11 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

15. The method of claim 11 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

16. The method of claim 11 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

17. The method of claim 16 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, intramuscular, aerosol topical or bronchoscopic delivery.

18. The method of claim 11 wherein said respiratory disorder is in the group comprised of all subsets of obstructive lung disease, including emphysema, asthma, bronchitis, fibrosis, excessive mucous secretion, obstruction of air flow, and lung disorders resulting from post-surgical complications.

19. A method for relaxing gastrointestinal smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

20. The method of claim 19 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

21. The method of claim 19 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

22. The method of claim 19 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

23. The method of claim 19 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

24. The method of claim 19 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

25. The method of claim 24 wherein said pharmaceutical composition is administered to a patient by a route comprising oral, sublingual, intravenous, topical, intramuscular, aerosol or endoscopic delivery.

26. A method for alleviating contraction or spasm of gastrointestinal smooth muscle associated with endoscopic procedures comprising, administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

27. The method of claim 26 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

28. The method of claim 26 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

29. The method of claim 26 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

30. The method of claim 26 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

31. The method of claim 26 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

32. The method of claim 31 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular, aerosol or endoscopic delivery.

33. A method for relaxing corpus cavernosum smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

34. The method of claim 33 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

35. The method of claim 33 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

36. The method of claim 33 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

37. The method of claim 33 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

38. The method of claim 33 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

39. The method of claim 38 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or aerosol delivery.

40. A method for the treatment or prevention of impotence, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

41. The method of claim 40 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

42. The method of claim 40 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

43. The method of claim 40 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

44. The method of claim 40 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

45. The method of claim 40 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

46. The method of claim 45 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or aerosol delivery.

47. A method for relaxing bladder smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

48. The method of claim 47 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

49. The method of claim 47 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

50. The method of claim 47 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

51. The method of claim 47 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

52. The method of claim 47 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

53. The method of claim 52 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or aerosol delivery.

54. A method for relaxing uterine smooth muscle, comprising administering a therapeutically effective amount of an S-nitrosothiol compound to an animal in need thereof.

55. The method of claim 54 wherein said S-nitrosothiol compound has the formula:

$$CH_3(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

56. The method of claim 54 wherein said S-nitrosothiol compound has the formula:

$$HS(CH_2)_xSNO$$

wherein:

X equals 2 to 20.

57. The method of claim 54 wherein said S-nitrosothiol compound has the formula:

$$ONS(CH_2)_xY$$

wherein:

X equals 2 to 20;

Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbomoyl, C_1C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl;

wherein aryl includes benzyl, naphthyl and anthracenyl groups.

58. The method of claim 54 wherein said S-nitrosothiol compound is selected from the group consisting of S-nitroso-N-acetylcysteine, S-nitroso-glutathione, S-nitroso-cysteine, S-nitroso-homocysteine, S-nitroso-penicillamine and S-nitroso-captopril.

59. The method of claim 54 wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

60. The method of claim 59 wherein said pharmaceutical composition is administered to an animal by a route comprising oral, sublingual, intravenous, topical, intramuscular or aerosol delivery.

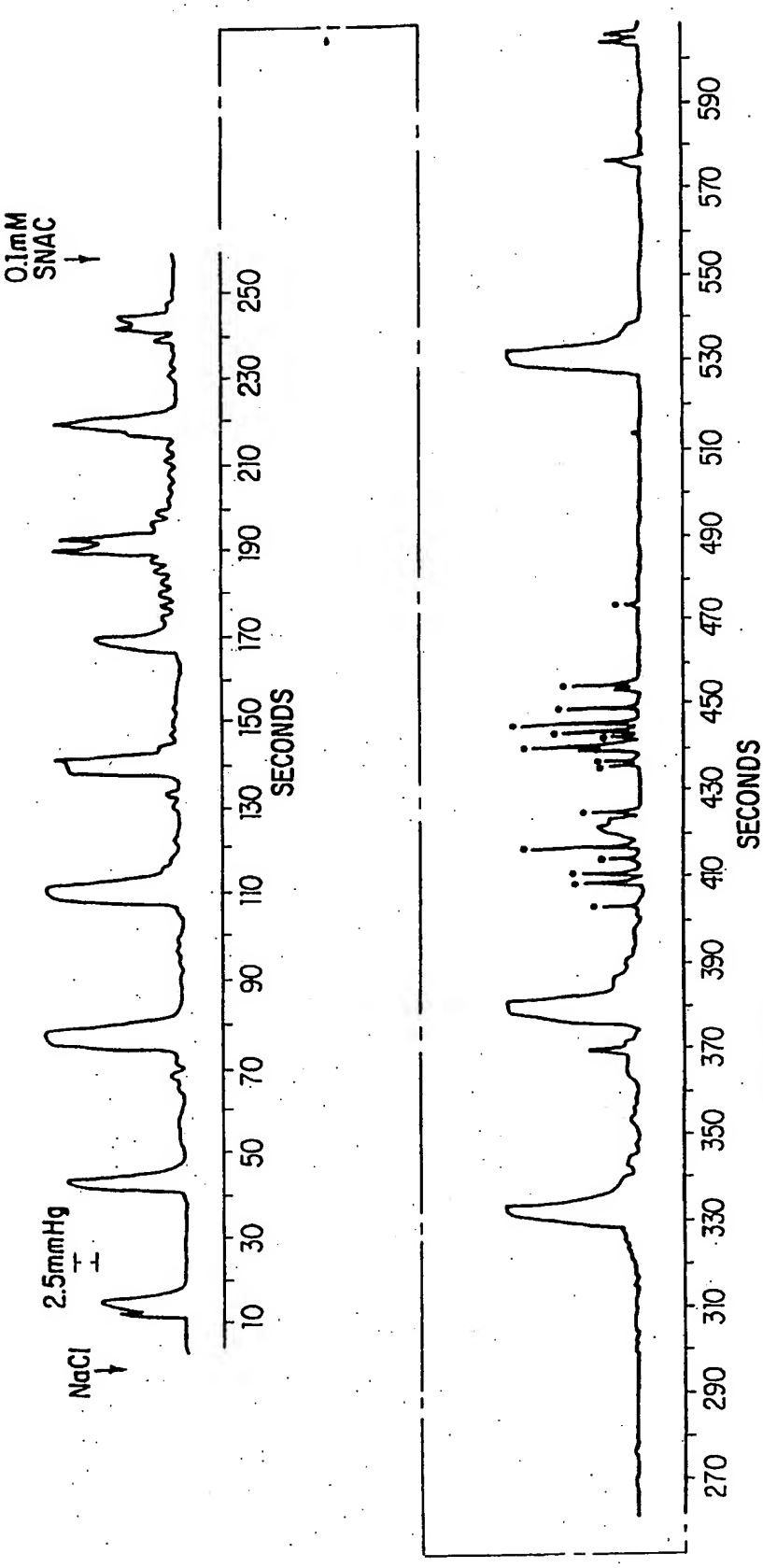
0.1mM
SNAC
2.5mmHg
NaCl
10
30
50
70
90
110
130
150
170
190
210
230
250
SECONDS
270
290
310
330
350
370
390
410
430
450
470
490
510
530
550
570
590
SECONDS
• = ARTIFACT

FIG. 1

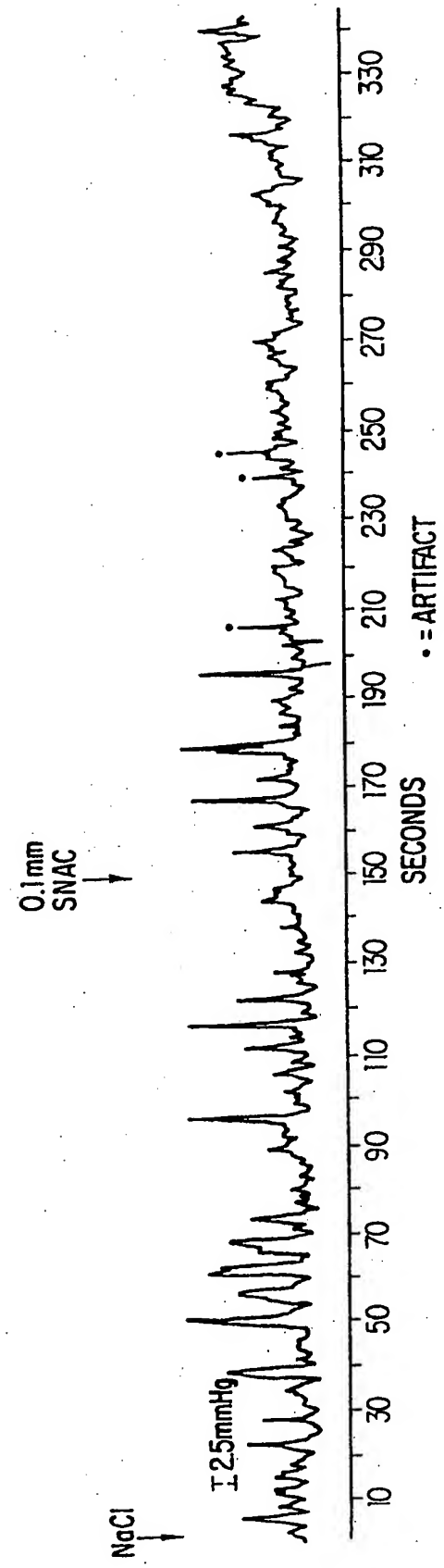
0.1mm
SNAC
NaCl
±2.5mmHg
10
30
50
70
90
110
130
150
170
190
210
230
250
270
290
310
330
SECONDS
• = ARTIFACT

FIG. 2

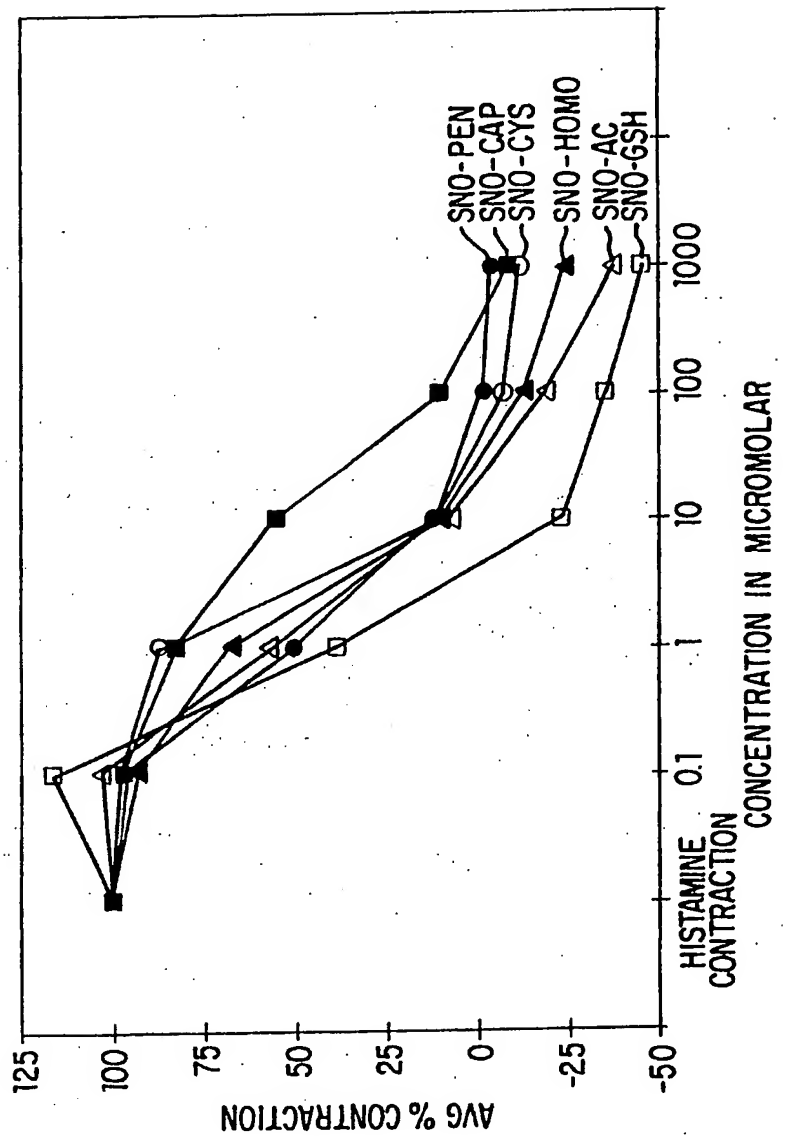
SNO-PEN
SNO-CAP
SNO-CYS
SNO-HOMO
SNO-AC
SNO-GSH
125
100
75
50
25
0
-25
-50
AVG % CONTRACTION
HISTAMINE CONTRACTION
0.1
1
10
100
1000
CONCENTRATION IN MICROMOLAR

FIG. 3

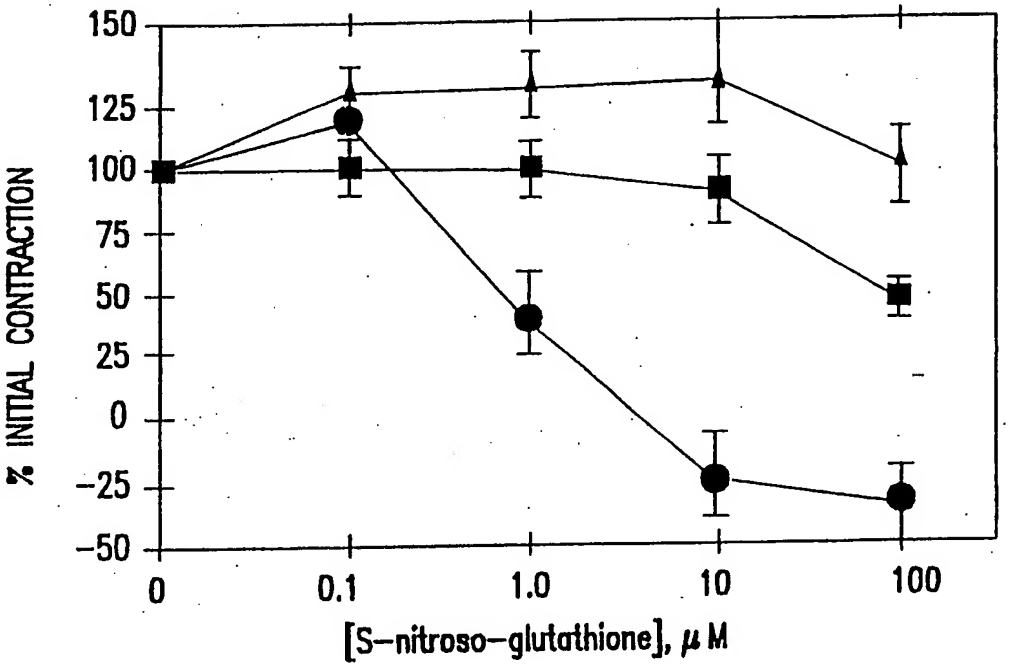
150
125
100
75
50
25
0
−25
−50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S−nitroso−glutathione], μM

FIG.4A

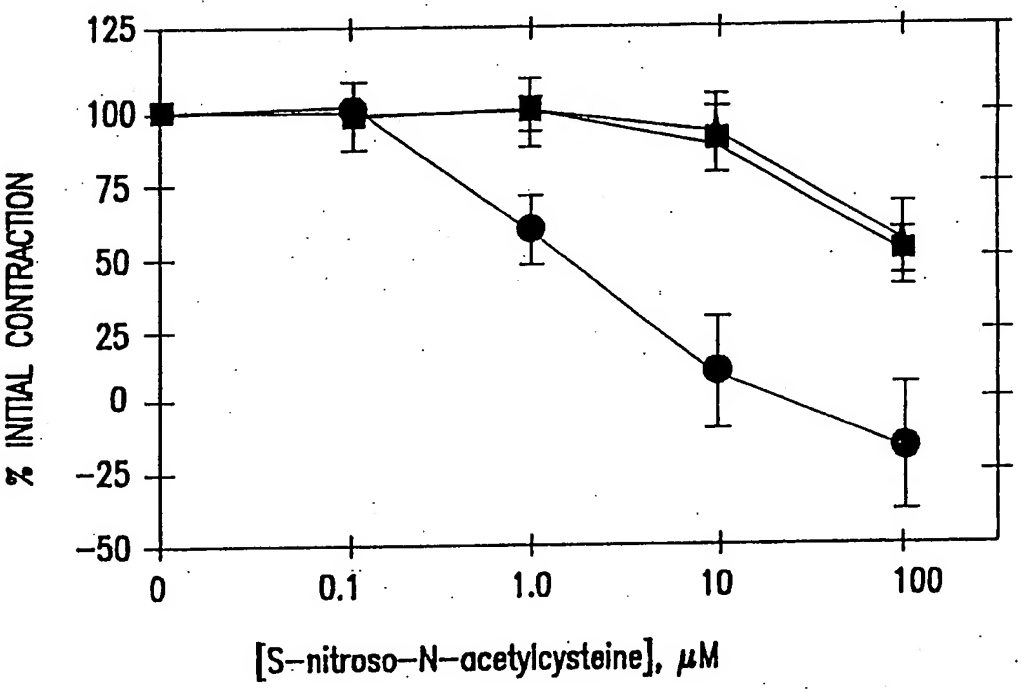
125
100
75
50
25
0
−25
−50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S−nitroso−N−acetylcysteine], μM

FIG.4B

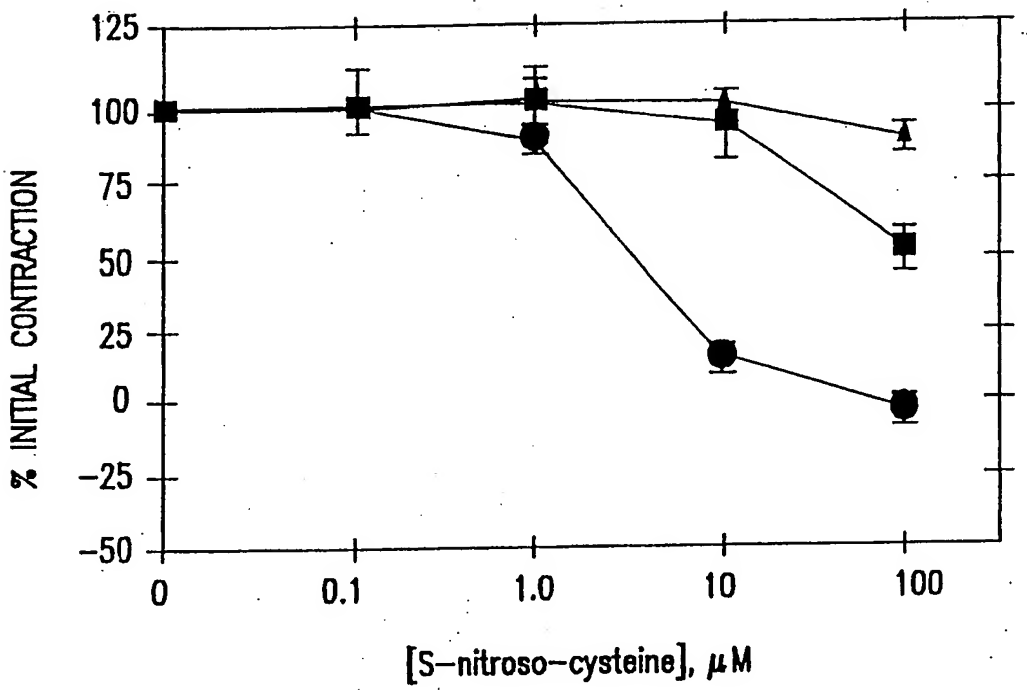
125
100
75
50
25
0
-25
-50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S-nitroso-cysteine], μM

FIG.4C

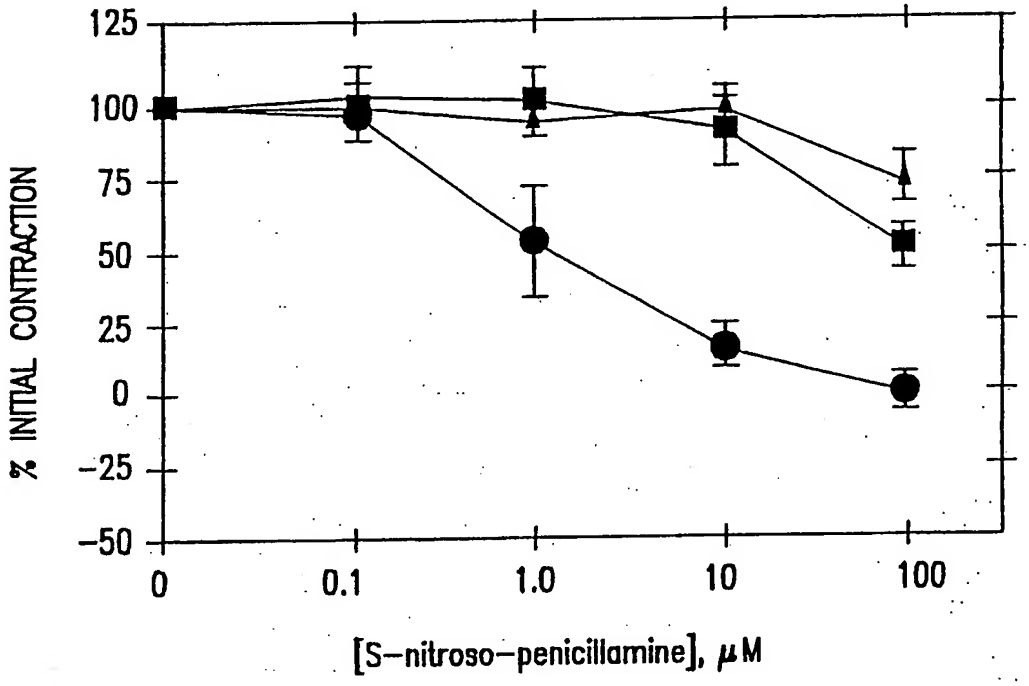
125
100
75
50
25
0
-25
-50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S-nitroso-penicillamine], μM

FIG.4D

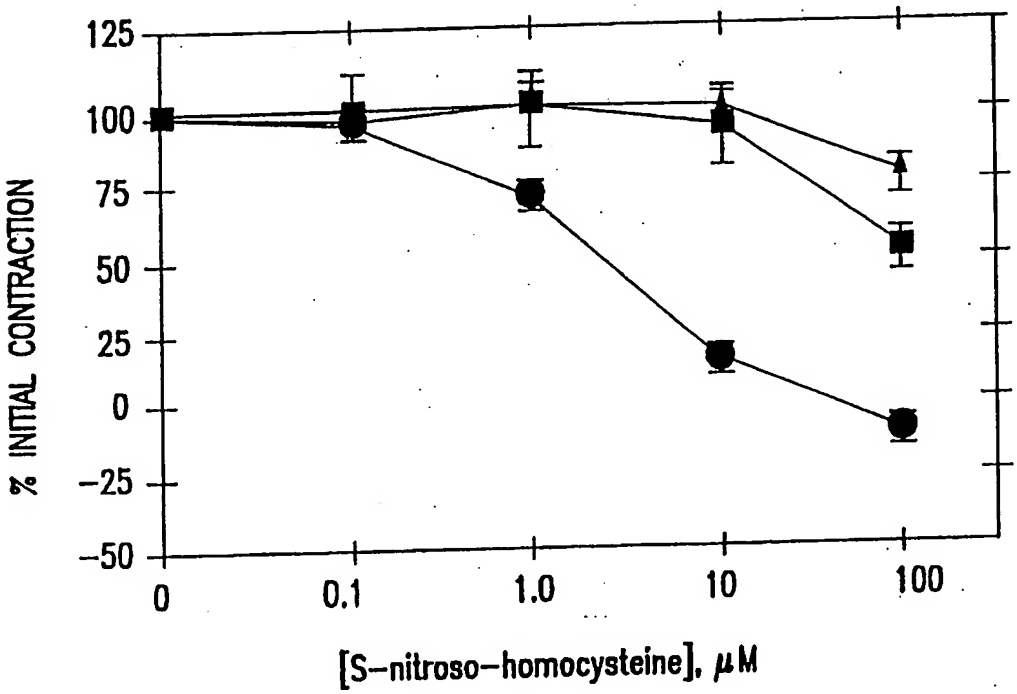
125
100
75
50
25
0
−25
−50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S−nitroso−homocysteine], μM

FIG.4E

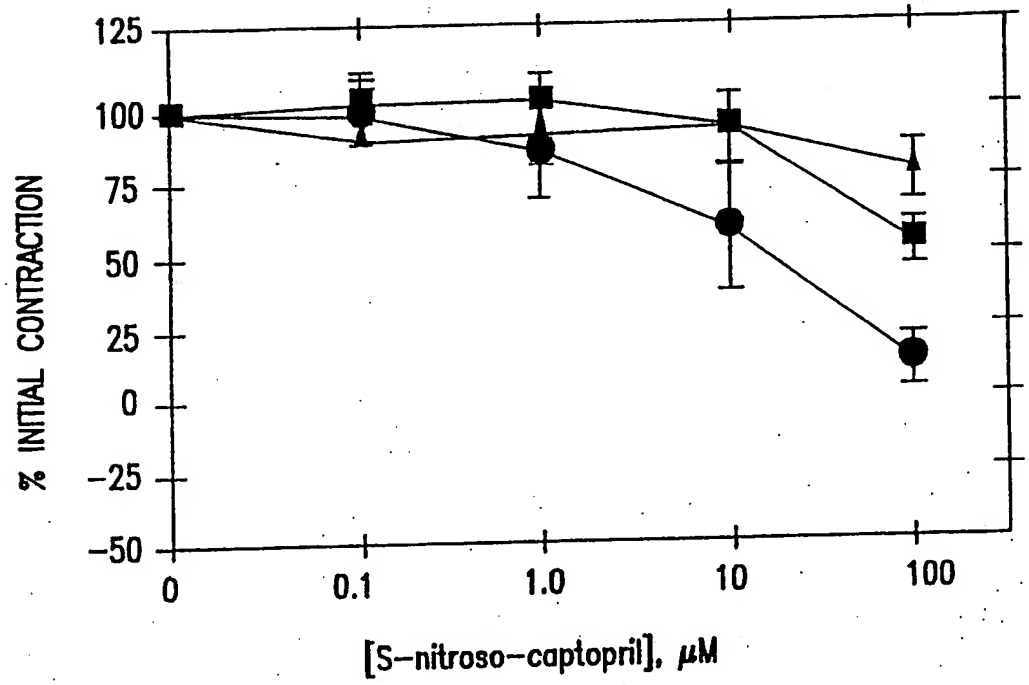
125
100
75
50
25
0
−25
−50
% INITIAL CONTRACTION
0
0.1
1.0
10
100
[S−nitroso−captopril], μM

FIG.4F

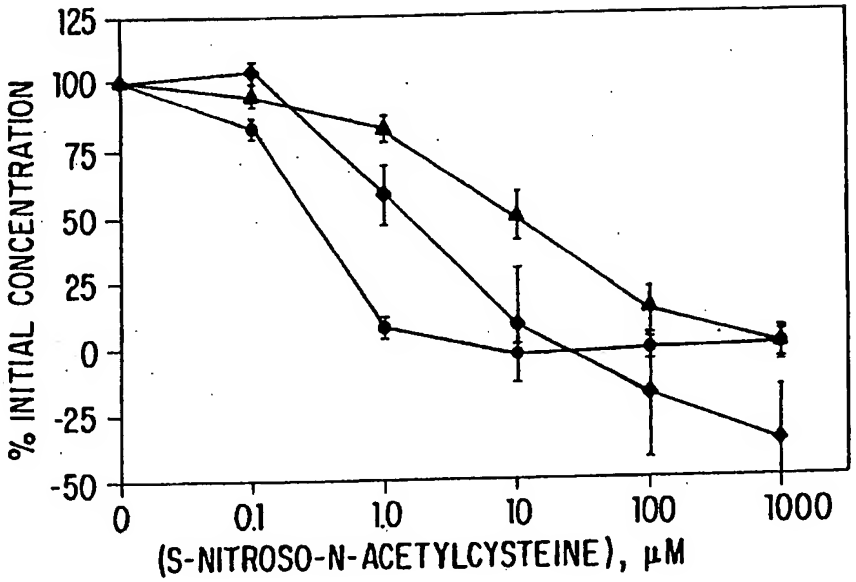
125
100
75
50
25
0
-25
-50
% INITIAL CONCENTRATION
0
0.1
1.0
10
100
1000
(S-NITROSO-N-ACETYLCYSTEINE), μM

FIG. 5A

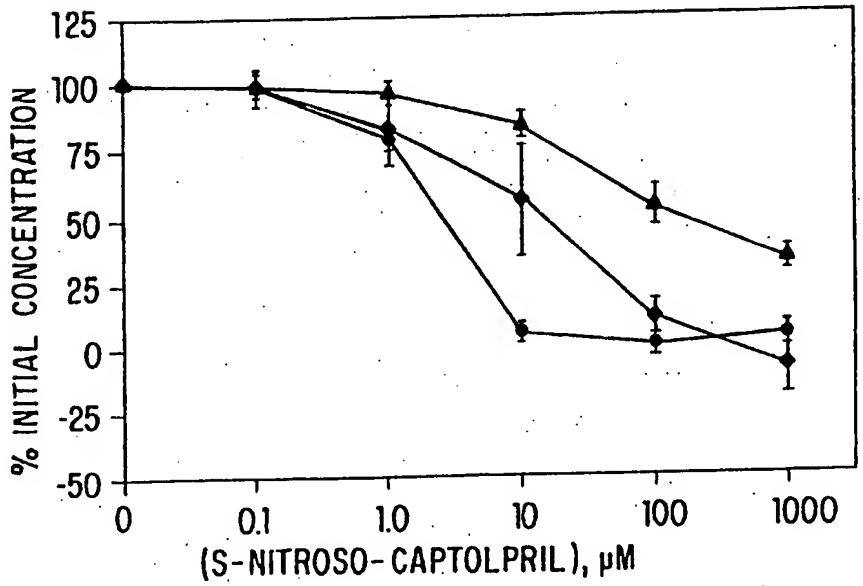
125
100
75
50
25
0
-25
-50
% INITIAL CONCENTRATION
0
0.1
1.0
10
100
1000
(S-NITROSO-CAPTOLPRIL), μM

FIG. 5B

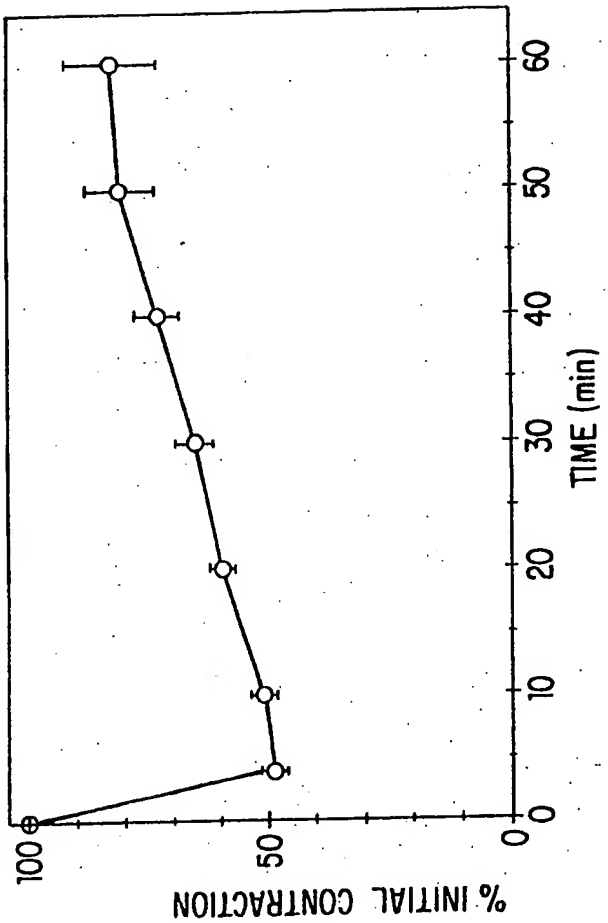

FIG. 6

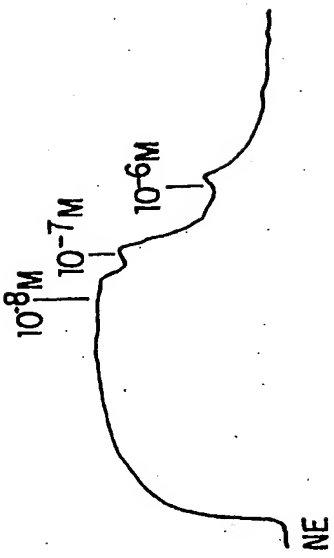

FIG. 7A

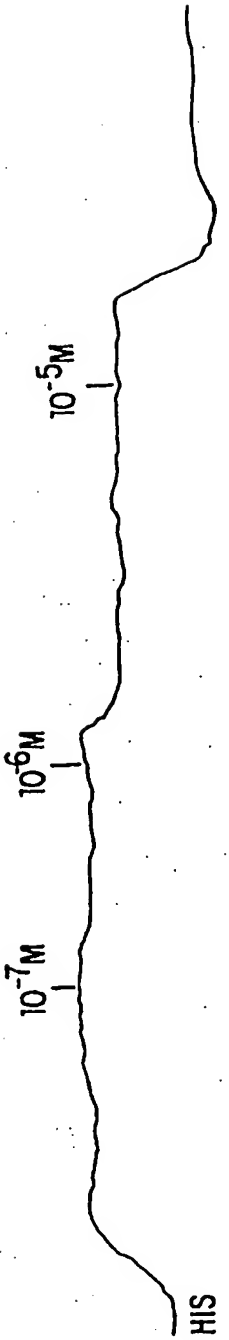

FIG. 7B

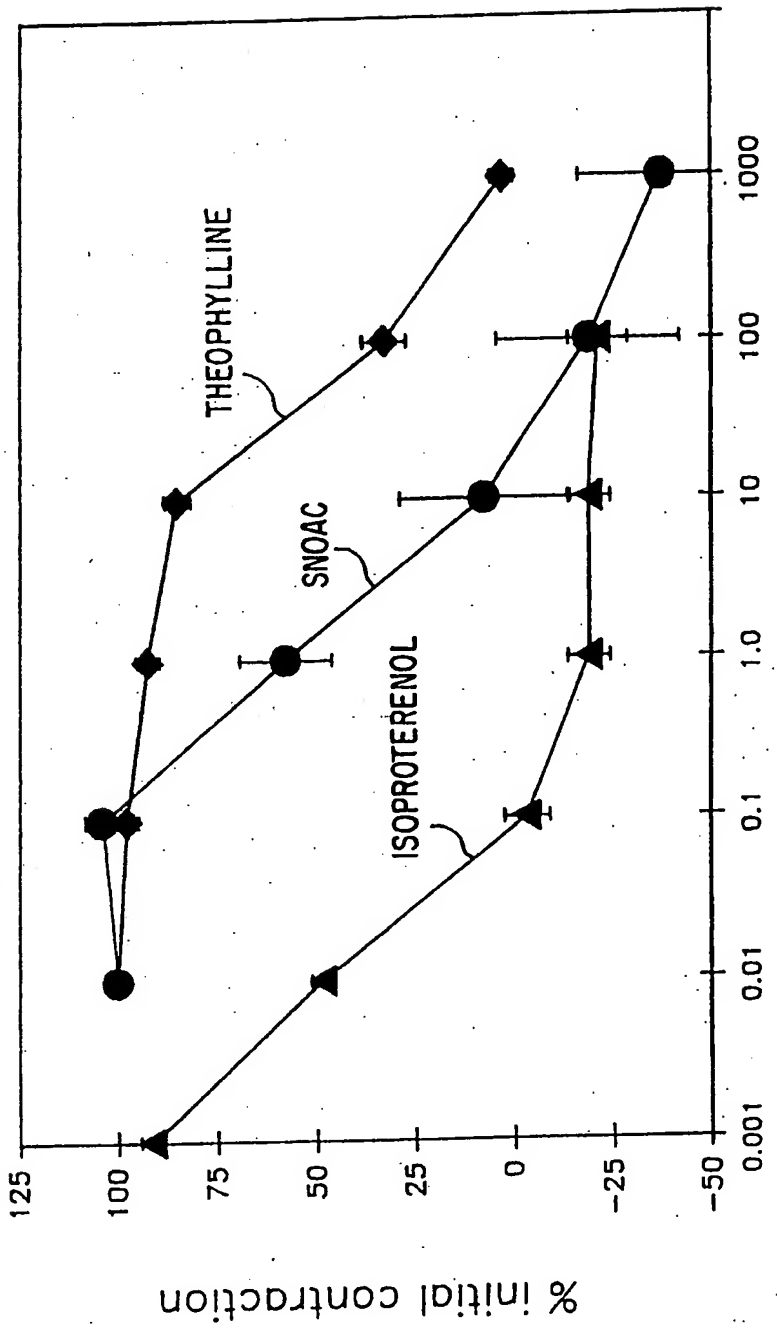
THEOPHYLLINE
SNOAC
ISOPROTERENOL
% initial contraction
125
100
75
50
25
0
-25
-50
0.001
0.01
0.1
1.0
10
100
1000
[Relaxant], μM

FIG. 8

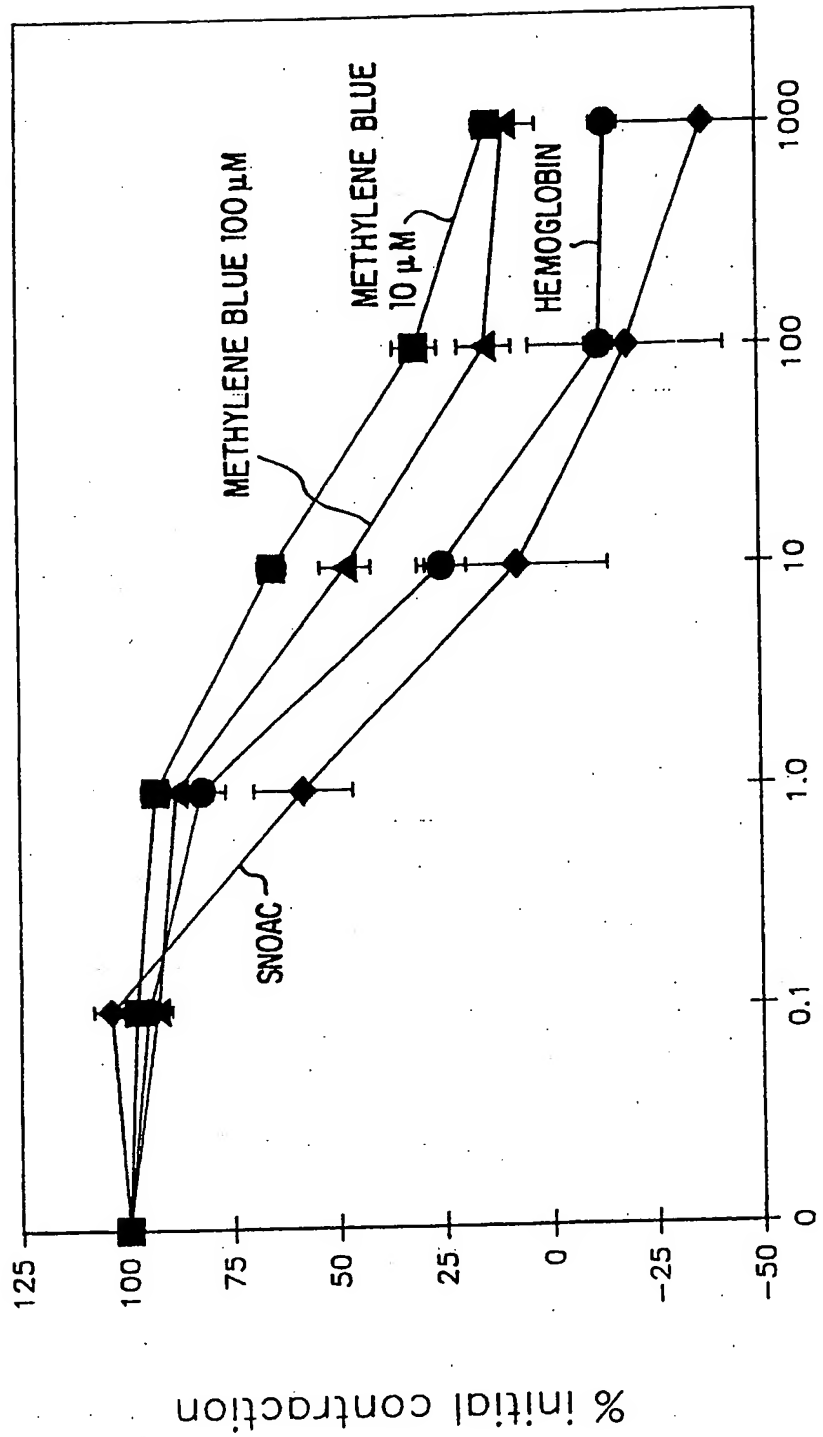
METHYLENE BLUE 100 µM
METHYLENE BLUE 10 µM
HEMOGLOBIN
SNOAC
125
100
75
50
25
0
−25
−50
0
0.1
1.0
10
100
1000
% initial contraction
[S−Nitroso−N−acetylcysteine], µM

FIG. 9

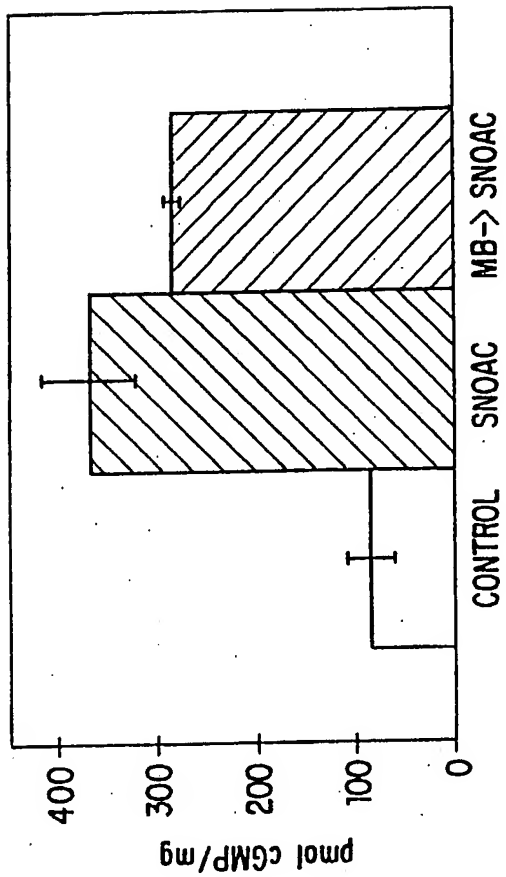

FIG. 10

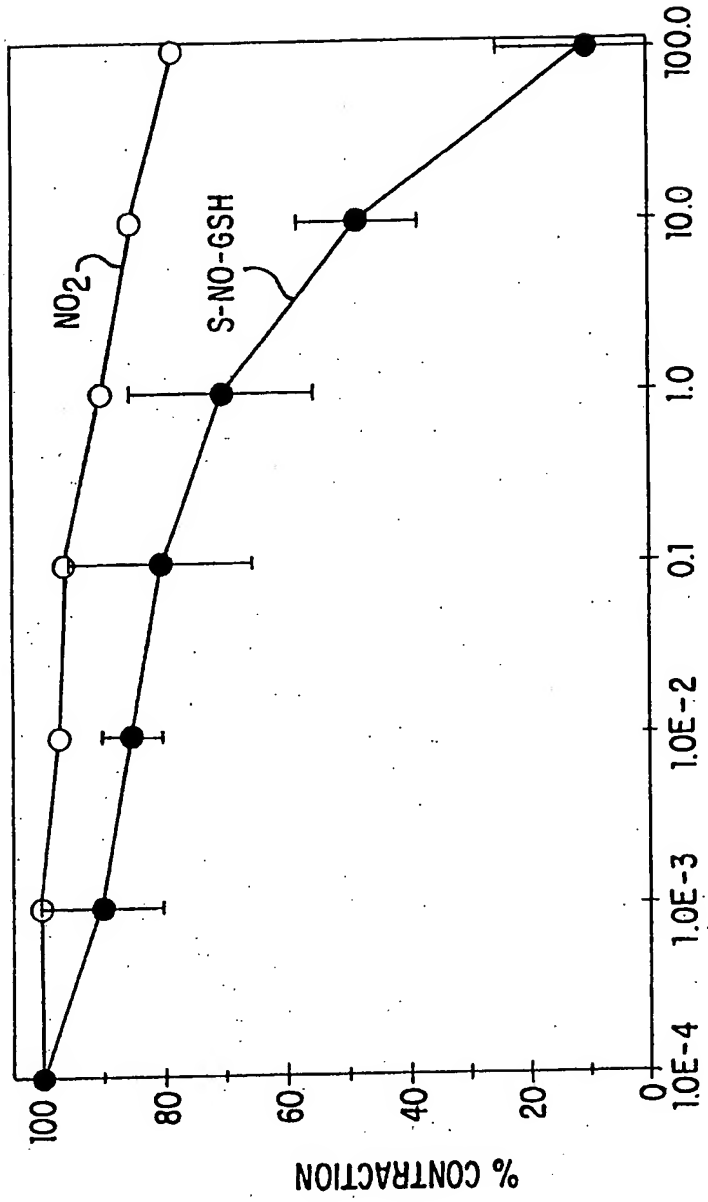
NO2
S-NO-GSH
100.0
10.0
1.0
0.1
1.0E-2
1.0E-3
1.0E-4
(RELAXANT), μM
100
80
60
40
20
0
% CONTRACTION

FIG. 11

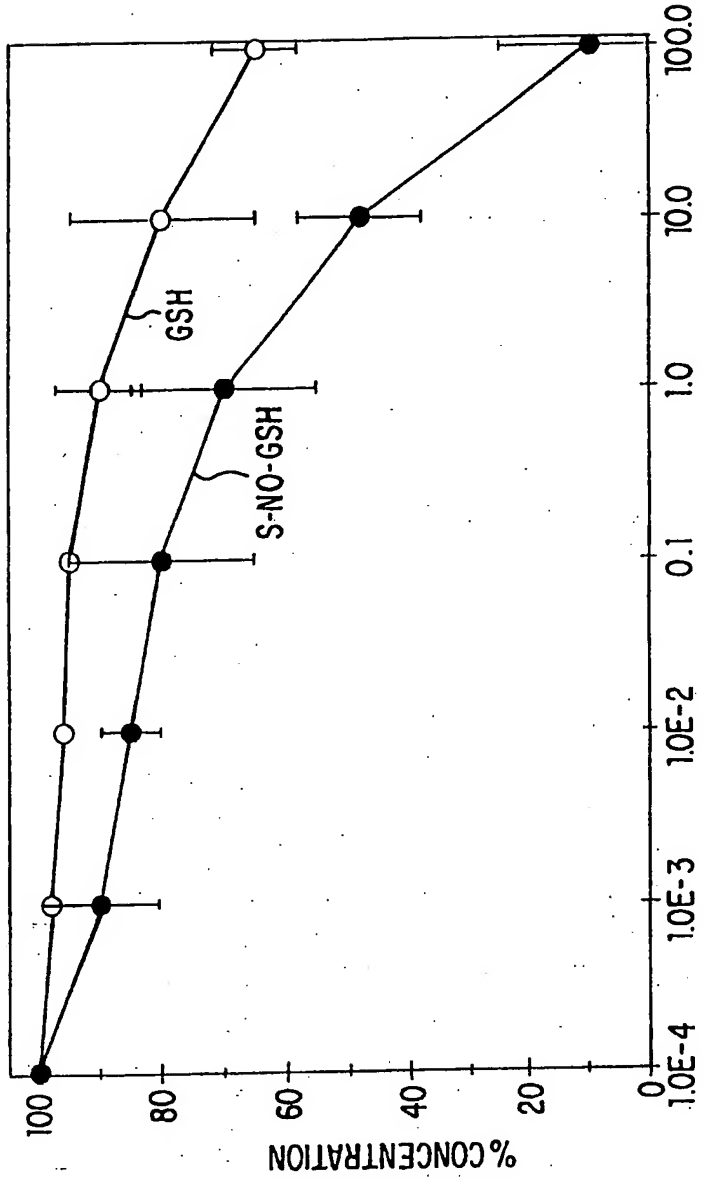
100.0
10.0
1.0
0.1
1.0E-2
1.0E-3
1.0E-4
(RELAXANT), μM
GSH
S-NO-GSH
100
80
60
40
20
0
% CONCENTRATION

FIG. 12

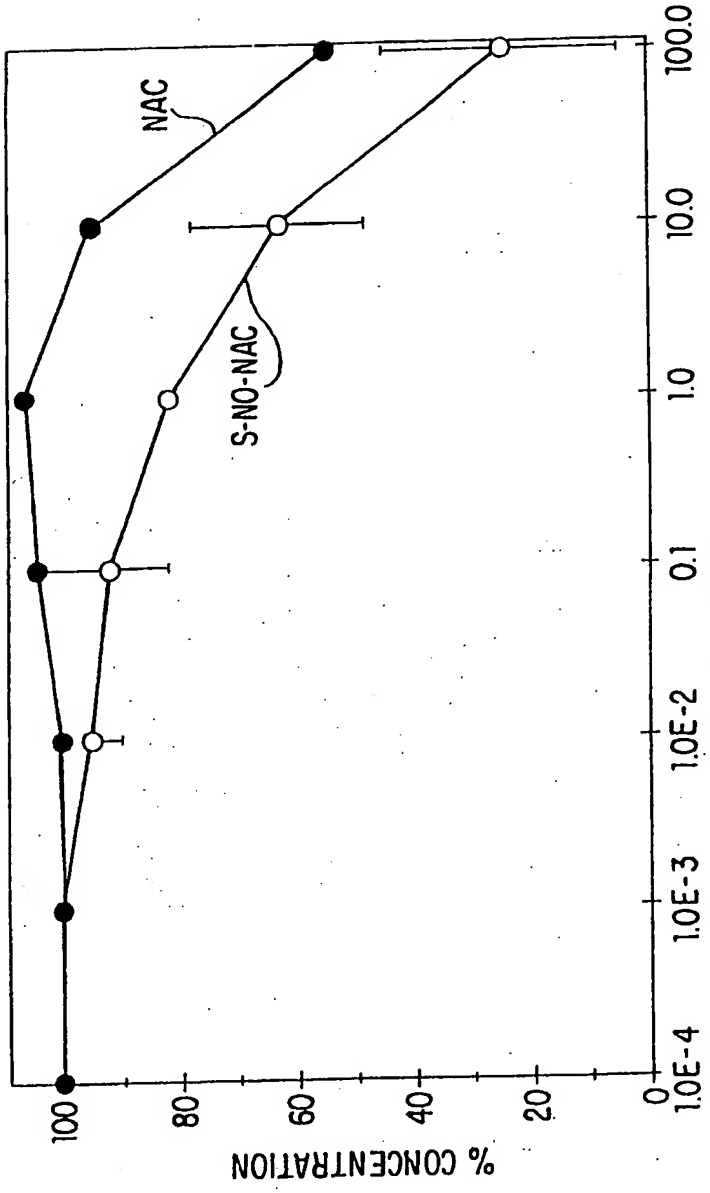
NAC
S-NO-NAC
100.0
10.0
1.0
0.1
1.0E-2
1.0E-3
1.0E-4
0
(RELAXANT), μM
100
80
60
40
20
% CONCENTRATION

FIG. 13

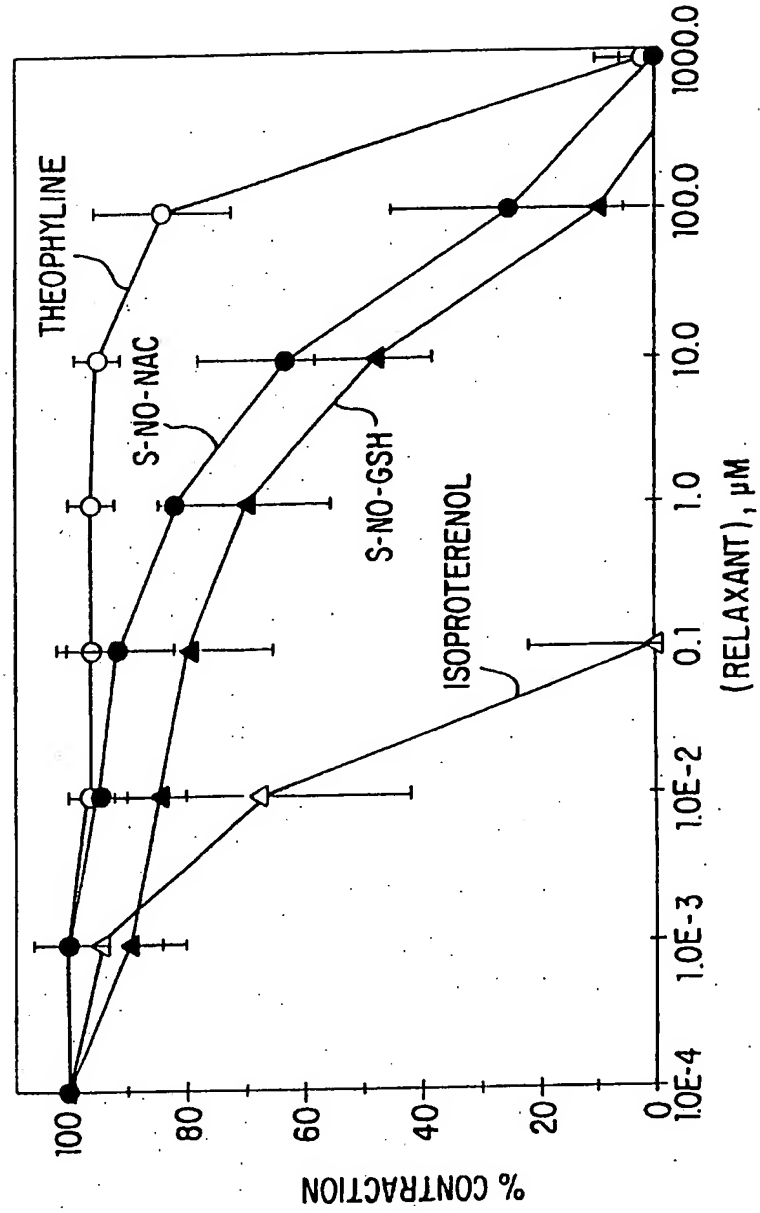
THEOPHYLINE
S-NO-NAC
S-NO-GSH
ISOPROTERENOL
% CONTRACTION
100
80
60
40
20
0
1.0E-4
1.0E-3
1.0E-2
0.1
1.0
10.0
100.0
1000.0
(RELAXANT), μM

FIG. 14

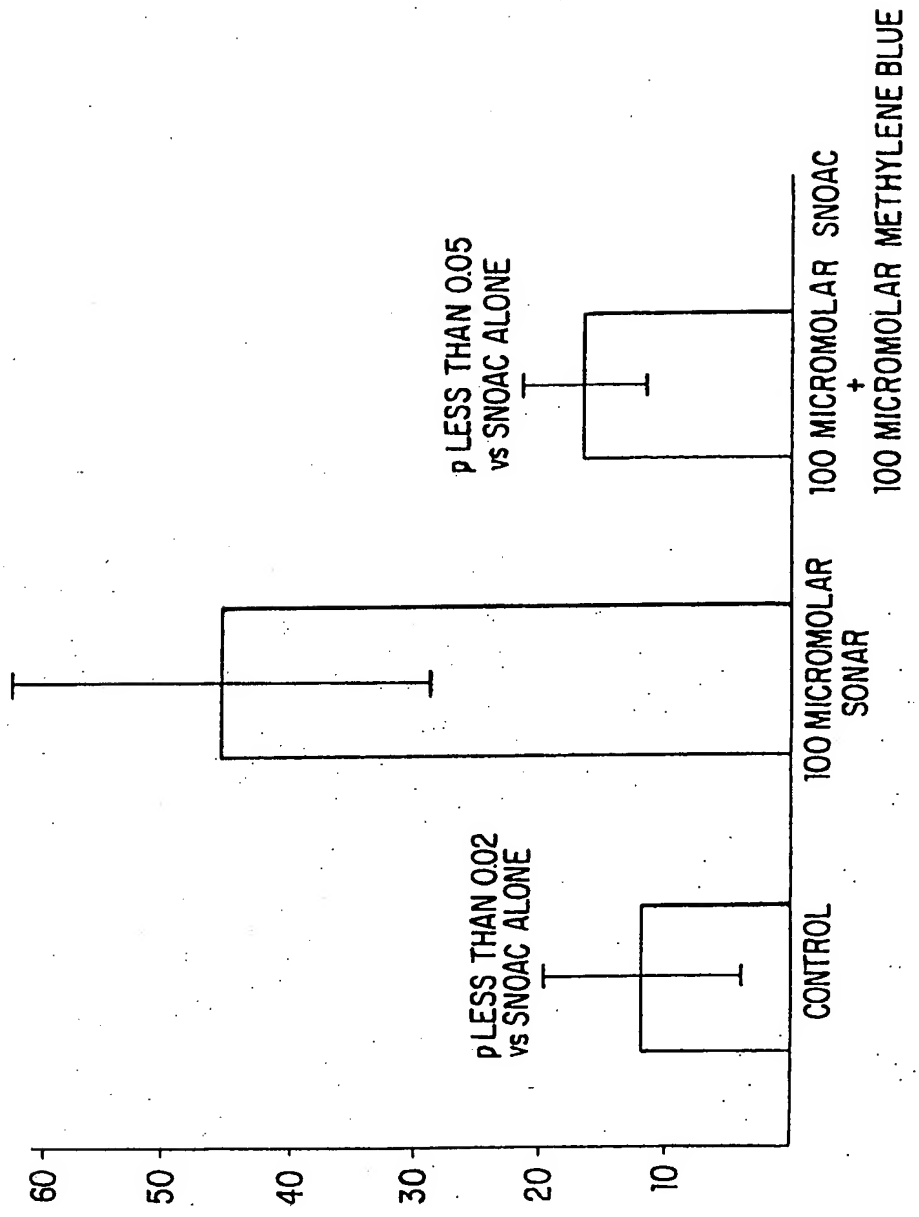
60
50
40
30
20
10
p LESS THAN 0.05
vs SNOAC ALONE
p LESS THAN 0.02
vs SNOAC ALONE
CONTROL
100 MICROMOLAR
SONAR
100 MICROMOLAR SNOAC
+
100 MICROMOLAR METHYLENE BLUE

FIG. 15